ANATOMICAL AND PHYSIOLOGICAL EVIDENCE FOR THE ROLE OF EXTRAOCULAR MUSCLE PROPRIOCEPTION IN THE CONTROL OF GAZE

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Abstract

The role of extraocular muscle proprioception in the control of gaze has been a subject of controversy for the past century. Generally, eye position signals involved in gaze control have been ascribed to internal copies of motor commands, often described as corollary discharges. In the last few years, however, a number of studies have provided strong evidence that afferent signals from proprioceptors in the extraocular muscles do affect the control of eye movements and visual localization in many animals, including Man.

The present study has investigated the effect of imposed eye movement on the electromyographic activity of dorsal neck and extraocular muscles and on movements of the contralateral eye, using the electrooculogram, during vestibular stimulation in the decerebrate pigeon. Imposing movements on one eye at saccadic velocities produced large modulations in the activity of dorsal neck and extraocular muscles which were dependent on the parameters of the imposed eye movement. Thus the largest effects on dorsal neck or extraocular muscle activity were seen when movement was imposed on the eye in the opposite direction to that produced by a compensatory vestibuloocular reflex, i.e. when a contracting extraocular muscle was stretched. Slow, sinusoidal imposed eye movements that mimicked the slow phase of the vestibuloocular reflex, but with amplitude and velocity errors, produced systematic changes in neck and eye muscle responses that were closely correlated to the peak eye velocity imposed. Similarly the movement of the contralateral eye was decreased by increasing amplitudes and velocities of this slow imposed eye movement in a manner that appeared to correct for the errors in the imposed eye movement which would tend to maintain the direction of gaze.

Section of the ophthalmic branch of the trigeminal nerve, the presumed pathway of extraocular muscle afferent fibres, abolished the effects of imposed eye movement on the activity of dorsal neck and extraocular muscles and on movements of the contralateral eye, strongly suggesting that the effects of imposed eye movement were mediated by extraocular muscle afferent signals. Section of the ophthalmic branch of the trigeminal nerve also disrupted the slow phase of the vestibuloocular reflex of the ipsilateral eye and caused instability of the ipsilateral eye at rest. This was strikingly similar to the effects seen by others in the cat and rabbit following section of the same nerve.

Injection of the anatomical tracer, horseradish peroxidase conjugated to wheatgerm agglutinin, into the extraocular muscles of one eye demonstrated an anatomical substrate for the observed physiological effects of imposed eye movement. Labelled neuronal cell somata were found in a restricted portion of the ipsilateral trigeminal ganglion. Labelling of these neurones was prevented by section of the ophthalmic branch of the trigeminal nerve, confirming that the ophthalmic branch contains afferent fibres subserving extraocular muscle proprioception. The central projections of extraocular muscle afferent neurones were consistently found in a restricted portion of the external cuneate nucleus. This nucleus, in the pigeon, receives terminals from trigeminal primary afferent neurones via the lateral trigeminal tract. It is known in the monkey that neck muscle afferent fibres project to both the cuneate and external cuneate nuclei. It is possible that the labelled terminals

in the external cuneate nucleus represent a site of interaction between extraocular muscle afferent signals and neck muscle afferent signals.

The results show that extraocular muscle afferent signals have striking effects on the electromyographic activity of dorsal neck and extraocular muscles during vestibular stimulation and strongly suggest that these signals are involved in head-eye coordination. The effects of section of the ophthalmic branch of the trigeminal nerve on the movement of the ipsilateral eye suggest that extraocular muscle proprioceptive afferent signals play a major role in the control and stability of eye movements. Finally, the results strongly support the growing view that extraocular muscle afferent signals play an important role in gaze control and that current models of gaze control are in need of revision.

Declaration

This thesis has been composed by myself and the work within it was carried out by myself, under the supervision of Professor I.M.L. Donaldson in the Department of Pharmacology, University of Edinburgh between October 1991 and September 1994.

Some of the results reported here have been published .

Published Papers

Hayman M.R., Knox P.C., Dutia M.B. and Donaldson I.M.L. (1993) Effects of extraocular muscle afferent signals on the electromyogram of pigeon neck muscles during the vestibulo-collic reflex. *J. Physiol.* **459**, 458P.

Hayman M.R., Dutia M.B. and Donaldson I.M.L. (1993) Extraocular muscle afferent signals affect the activity of neck muscles in the pigeon. *Brain Res. Assoc. Abstr.* **10**, 49.

Hayman M.R., Dutia M.B. and Donaldson I.M.L. (1993) Extraocular muscle afferent signals modify the activity of neck muscles during the vestibulo-collic reflex. *Perception [Suppl]* **22**, 72.

Hayman M.R., Dutia M.B. and Donaldson I.M.L. (1993) Afferent signals from pigeon extraocular muscles modify the activity of neck muscles during the vestibulocollic reflex. *Proc. Roy. Soc. Lond. B* **254**, 115-122.

CHAPTER 1. REVIEW OF EXTRAOCULAR MUSCLE PROPRIOCEPTION

1.1 INTRODUCTION

Information about the direction in which our eyes are pointing is necessary in order to assess the direction of a visible object and to perform any form of spatially oriented behaviour. It is known that the retinal position of an image alone does not provide sufficient spatial information (Sherrington, 1918; Skavenski, 1976). Visual localization and visuomotor activities are thus considered to require a combination of signals indicating eye position with respect to the head. However, the source of such an extraretinal eye position signal has been a subject of controversy for over 100 years.

The two possible sources of the extraretinal eye position signal are generally described as 'inflow' and 'outflow'. The inflow of eye position information from extraocular muscle (EOM) proprioceptors, championed by Sherrington, has not been considered to play a major role in the control of eye movement or in visual localization (Carpenter, 1988; MacKay, 1972; Steinbach, 1986). Outflow, first suggested by Helmholtz, and formalised by the theories of corollary discharge (Sperry, 1950) and efference copy (Von Holst and Mittelstaedt, 1950) has generally been considered to provide the only useful extraretinal signal (Steinbach, 1987). This view has gained ascendancy not through experimental proof of the lack of an effective proprioceptive signal, or the presence of an accurate outflow signal, but through the lack of a monosynaptic stretch reflex, the believed absence of position sense in the eye and the assertion of oculomotor modellers that such a signal was unnecessary.

However, the extraocular muscles contain numerous sensory endings which have been shown to respond to stretch, and responses to EOM stretch have been noted in many brainstem and cortical areas. A major problem has been to ascribe a particular function to inflow from EOM proprioceptors. As recently as 1988 Carpenter could justifiably state:

> "After more than a century of diligent research by physiologists we have to admit that we have no clear idea at all what these receptors are actually *for*."

The evidence for the presence of stretch receptors within the EOM, their properties and functions is scattered through one hundred years of literature. While there have been short summaries of the current and historical literature, (Whitteridge, 1960; Bach-y-Rita, 1971; Sivak, 1983; Steinbach, 1987) there has not been a

comprehensive review of EOM proprioception in the last twenty years. Yet during this time a number of elegant and important studies have been published which strongly suggest EOM proprioception plays a functional role in visuomotor behaviour and control. The following short review does not attempt to be exhaustive, but will summarise much of the preceding 100 years' work on the EOM proprioceptors.

1.2 MORPHOLOGY OF THE EXTRAOCULAR MUSCLES

The organisation and morphology of fibre types in the EOM have been described in a number of excellent reviews (Peachey, 1971; Chiarindini and Davidowitz, 1979; Spencer and Porter, 1988) so only a brief summary of current knowledge will be attempted here. Spencer and Porter (1988) comment that "The presence of these six principal [extraocular] muscles, the four recti (superior, inferior, medial and lateral) and two obliques (superior and inferior), is rather constant, with few exceptions across the vertebrate classes". Differences in the insertions and actions of the EOM between different vertebrate species are predominantly due to the orientation of the semicircular canals (Ezure and Graf, 1984; Simpson and Graf, 1981) and the presence of lateral or forward facing eyes. While the two horizontal recti are unchanged between lateral and forward facing eyes, the other four EOM show considerable differences in their secondary actions.

Kato (1938) described the EOM as having two distinct regions, an outer, orbital layer and an inner, global layer. Two basic types of muscle fibre, characterised on the basis of their histological appearance were described as *Fibrillenstruktur* and *Felderstruktur* (Kruger, 1929; Siebeck and Kruger, 1955). *Fibrillenstruktur* muscle fibres resemble the twitch muscle fibres seen in other skeletal muscle with a well developed sarcoplasmic reticulum in close contact with regularly spaced fibrils and large single nerve endings on each fibre (described as *en plaque*). *Felderstruktur* muscle fibres, on the other hand are almost unparalleled amongst skeletal muscle fibres in mammals, resembling the slow muscle fibres found in amphibians and birds. Thus *Felderstruktur* muscle fibres have a poorly developed sarcoplasmic reticulum, with irregular masses of bunched up fibrils and multiple endings (described as *en grappe*) with quite unspecialised subsynaptic regions. The classification of EOM muscle fibres on such histological grounds was corroborated by findings that although the EOM are the fastest contracting fibres found in

mammals (Cooper and Eccles, 1930) suggestive of twitch muscle fibres, they are also extremely sensitive to acetylcholine-induced contractions (Duke-Elder and Duke-Elder, 1930) which is typical of multi-innervated slowly contracting muscle fibres. The division of the EOM into two distinct layers (global vs orbital) and two basic muscle fibre groups (*Fibrillenstruktur* vs *Felderstruktur*) is still the classification used for describing the different muscle fibres subsequently found within the EOM (Spencer and Porter, 1988).

Histochemical and ultrastructural analysis of the EOM initially confirmed the division of EOM fibres into two classes (Carpenter, 1988), however careful studies have shown that the fast twitch (Fibrillenstruktur) muscle fibres can be further divided into a small number of different classes. Peachey (1971) suggested four or five classes using criteria such as diameter, position, fibril arrangement, histochemical features and number of mitochondria. Such divisions have since been confirmed by other workers (Maier et al, 1972; Alvarado and van Horn, 1975 and Pachter, 1982, 1983). The most recent and complete classification was described by Spencer and Porter (1988). They used a large number of histochemical and ultrastructural findings to divide EOM fibres into 6 different types; two in the orbital layer and four in the global layer. Four different singly-innervated, fast, twitch fibres were identified in agreement with previous authors, and two types of multiply-innervated slow, tonic fibres, again in agreement with previous authors (Peachey, 1971; Lennerstrand, 1974; Alvarado and van Horn, 1974 and Chiarindini and Davidowitz, 1979). Thus the orbital layer contains two types of muscle fibres, an orbital singly-innervated muscle fibre and an orbital multiply innervated muscle fibre. The global layer contains four types of muscle fibre, a global red singly-innervated muscle fibre, a global intermediate singly-innervated muscle fibre, a global pale singly-innervated muscle fibre and a global multiply innervated muscle fibre.

1.3 MORPHOLOGY OF EOM PROPRIOCEPTORS

The presence of muscle spindles in the EOMs of animals appears to follow no rhyme or reason. Whilst Sherrington showed that muscle spindles were the major proprioceptors of skeletal muscles, he was unable to find any spindles in the EOMs of the rhesus macaque monkey, rabbit or cat. Many scientists have looked for spindles and other proprioceptors in EOMs: Buzzard (1908) reports finding in Man, "the very occasional presence of easily recognised muscle-spindles in ocular muscles" Cilimbaris (1910) showed muscle spindles to be present in sheep, ox, deer, goat and wild pig, but not in domestic pig, horse, cat, dog, fox, rabbit, hare and rat. Wohlfart (1935) reported numerous spindles in the EOM of calves; Sutton (1915) states the presence of spindles in the embryonic domestic pig, but comments that certain structures disappear soon after birth and Greene and Jampel (1966) report finding a few spindles in macaque EOM. Muscle spindles in human EOM were not positively identified until Cooper and Daniel's 1949 paper, but this finding has since been confirmed a number of times (Sunderland, 1949; Merillees et al, 1950; Maier, 1974; Ruskell, 1989). The most comprehensive survey of spindles in EOM is that of Maier (1974), who tested EOM from 27 different species, including amphibia, birds, rodents, carnivores and primates. Maier's work confirms the results of previous studies (see Table I): only a few spindles are present in higher primates (macaque and chimpanzee), considerably more in human EOM (but see later), but the largest number of spindles are found in the EOM of the artiodactyl branch of the ungulata (camel, cattle, deer, elk, giraffe, goat, gnu, pig, sheep and wild pig). The only report of spindles that does not appear to fit into this neat, if inexplicable, classification, is that of the presence of spindles in an unnamed strain of albino mouse (Mahran and Sakla, 1965). This result has not been successfully repeated (Maier, 1974).

Close examination of the spindles found in human EOM and sheep show very large differences. While the spindles found in sheep EOM have a 'classical' histological appearance (Harker, 1972) and have been shown to function similarly (Browne, 1975), those in human EOM , in the words of Ruskell (1989), "differ from all other muscle spindles studied so far." Ruskell's careful ultrastructural observations confirm and extend those of Cooper and Daniel (1949) who stated that in Man the spindles are "a smaller and more delicate end-organ than the comparable structure in the other somatic muscles" with the most striking differences being the "very much thinner capsule" and the fact that intra- and extrafusal muscle fibres do not differ very greatly in size. Ruskell comments that "Variations reported among mammalian spindles such as the different ratios of receptors, fibre diameters and

incidence of secondary endings are minor compared to the aberrant features of human EOM spindles...their setting suggests that they are expressions of spindle reorganisation imposed by degeneration." Perhaps even more surprisingly for a scientific community used to the supremacy of the muscle spindle as a proprioceptor comes the comment:

"Human EOM spindles have lost, wither (sic) by ageing or phylogenetically, the privilege of contractile chambers isolated by a fluid periaxial space from extrafusal fibre activity and sensory terminals are subject to the direct mechanical influences of anomalous intrafusal fibres. These, and the other departures from normal structure described, must jeopardise monitoring of muscle activity in the manner normally attributed to spindles and their capacity to provide useful proprioceptive information is questionable." (Ruskell, 1989).

Table 1.

ANIMAL	MUSCLE SPINDLES	MUSCULO-TENDINOUS CYLINDERS	FREE NERVE ENDINGS	REFERENCES
BONY				
FISHES	•	•	YES	Sabussow (1964)
Perch	•	•	YES	Ibid.
Pike	•	•	YES	Montgomery (1980)
Roach				
AMPHIBIANS				
Frog	•	•	YES	Maier, et al (1974)
REPTILES				
Snake	•	•	YES	Maier, et al (1974)
Salamander	•	•	YES	Ibid.
Turtle	•	•	YES	Ibid.
BIRDS				
Quail	NO	•	YES	Maier, et al (1971)
Pigeon	NO	•	YES	Ibid.
Sparrow	NO	•	YES	Ibid.
Canary	NO	•	YES	Ibid.

MAMMALS				
Loris	NO	•	•	Maier, DeSantis & Eldred (1974)
Mangabey	NO	YES	?	Maier, DeSantis & Eldred (1974)
Macaque	?	YES	?	Sherrington (1898); Greene (1966)
Chimpanzee	YES	•	?	Maier, DeSantis & Eldred (1974)
Man	YES	YES	?	Richmond (1984)
Rat	NO	•	YES	Daunicht (1985)
Albino	YES	•	•	Mahran & Sakla (1965)
mouse	NO	•	•	Maier, DeSantis & Eldred (1974)
Guinea pig	NO	YES	YES	Prince (1964), Cilimbaris (1910)
Rabbit	NO	•	•	Cilimbaris (1910)
Hare	NO	•	•	Maier, DeSantis & Eldred (1974)
Whale	NO	YES	•	Dogiel (1908)
Dog	NO	YES	•	Maier, DeSantis & Eldred (1974)
Fox	NO	•	•	Maier, DeSantis & Eldred (1974)
Bear	NO	YES	YES	Alvarado-Mallart (1979)
Cats	NO	•	•	Maier, DeSantis & Eldred (1974)
Cheetah	•	YES	•	Dogiel (1906)
Horse	YES	YES	•	Marchi (1882); Sutton (1915)
Pig	YES	YES	•	Maier, DeSantis & Eldred (1974)
Boar	YES	•	•	Crevatin (1902)
Camel	YES	•	•	Maier, DeSantis & Eldred (1974)
Giraffe	YES	•	•	Cilimbaris (1910)
Deer	YES	•	•	Maier, DeSantis & Eldred (1974)
Elk	YES	YES	•	Marchi (1882); Wohlfart (1935)
Cow	YES	•	•	Cilimbaris (1910); Winckler
Goat	YES	•	•	(1955)
Gnu	YES	NO	•	Maier, DeSantis & Eldred (1974)
Sheep	YES	NO	•	Harker (1972)

• represents this structure not mentioned in report. ? different authors report different findings

The rather odd distribution of muscle spindles among animals could be used as an argument against there being any afferent information relating to eye position if there were not a wealth of other suitable proprioceptors within the EOM of all animals so far examined. A number of putative sensory endings have been described

in animals whose EOM are devoid of spindles. Sir Charles Sherrington investigated the EOM sense organs and afferent fibres of the oculomotor nerves, in the rhesus macaque monkey. In a series of papers from 1898 Sherrington cut various combinations of the III, IV, V and VI cranial nerves in macaques and studied the EOM for evidence of degeneration of the "wealth of afferent fibres " within them. Tozer and Sherrington's 1910 paper is the culmination of this work. The overall conclusion of this paper was that: "The third, fourth and sixth cranial nerve pairs are therefore afferent-efferent, their afferents belonging to the receptive (sensorial) endings with which all the extrinsic eye-muscles are richly provided. The afferent divisions of these cranial nerves are, by their distribution exclusively proprio-ceptive...". It is also worth noting that a few sensorial musculo-tendinous organs of the extrinsic eye muscles were found to degenerate after cutting the ophthalmic branch of the trigeminal (V) nerve. The "receptive (sensorial) endings" described in the paper were both musculo-tendinous and intramuscular.

The musculo-tendinous sense organs pictured by Tozer and Sherrington were described by Marchi (1882) in cattle, pig, cat, rabbit and Man; Huber in rabbit (1899) and cat (1900); Crevatin (1902) in camel; Dogiel (1906) in cat, dog, horse, monkey and man; Pallot (1934) in cat and Sabussow (1964) in various amphibia. Musculotendinous cylinders have also been found in human EOM (Richmond, et al, 1984). Dogiel suggested that they resembled 'palisades' and they have since generally been called palisade endings. Ruskell (1978) showed that the 'palisade' endings terminate solely on slow, multi-innervated muscle fibres (Felderstruktur) within the global layer, in rhesus macaque monkey EOM and commented that the phrase 'palisade' ending was a rather poor description of the sensory ending: "because the axons form a sheaf with terminals located across the muscle fibre tip rather than a sheath attaching to the external surface". Ruskell suggested musculotendinous cylinder as a more suitable description, and these sensory structures shall be called such henceforth. The ultrastructural characteristics were shown to "clearly compare with the sensory endings of muscle spindles and contrast with motor terminals". The musculotendinous cylinders are similar in structure to the developing Golgi Tendon Organ (GTO) seen by Zelena and Soukup (1977) in the foetal rat. Ruskell's observations in the Rhesus monkey and his interpretation of the functional role of the musculotendinous cylinder was in close agreement with a study on the 'palisades' in the EOM of the cat by Pinçon-Raymond and Alvarado-Mallart (1979). Both studies suggest that the musculotendinous cylinder functions as a highly selective GTO and stress the recent findings from GTO receptors in the periphery that contraction of single muscle fibres, or a select few, produce afferent discharge and that such

stimulation is likely to be far more effective in eliciting an afferent response from the receptor than passive stretch of the whole tendon or muscle, with the diffusion of tension throughout the elastic tendon that this would produce (Houk and Henneman, 1967; Jami, 1992).

The other major putative sensory receptor that has been suggested is the free spiral ending seen in a number of animals devoid of spindles (e.g. Cooper and Fillenz, 1955, in the cat and Sas and Appeltauer in post-mortem human subjects, 1963). Ruskell (1984) has recently shown that the various forms of spiral nerve ending found in man and Rhesus monkey are in fact motor end plates (but see later). These nerve endings stained for acetylcholinesterase and had ultrastructural features of motor endings (e.g. a large, 60nm, synaptic cleft, boutons containing vesicles which were aggregated at the synaptic cleft and a basal lamina). The presence of GTO in Rhesus monkey EOM was also studied by Ruskell (1979) who came to the conclusion that they were few in number, unlike GTO seen in other skeletal muscles, were probably an aberrant development of musculotendinous cylinders, and they were "of little significance in the total sensory output".

In two studies the trigeminal ganglion has been injected with neuroanatomical tracers and the EOM examined for sensory receptors thus labelled. Daunicht (1985) injected HRP into the trigeminal ganglion of the rat and found 20-40 fibres in each of the EOM, some of which passed through the muscle and into the tendon (presumably musculotendinous cylinders). In each muscle Daunicht noted 1-3 fibres with a characteristic branching and meandering appearance. Billig (1991) injected the cat trigeminal ganglion with the tracers Fast Blue and Biocytin. In a thorough study of the EOM she noted palisade endings and four other intramuscular endings, including a simple spiral that she compares with those described by Cooper and Daniel (1955) and Sas and Appeltauer (1963). Thus Ruskell's finding that spiral endings are motor may represent only a portion of the spiral endings found within the EOM. One criticism of the two studies is that direct injections into the trigeminal ganglion will not fill proprioceptive afferent nerve fibres alone. Thus some of the endings found may represent sympathetic or pain fibres.

It is interesting to note that Harker (1972) reports finding no musculotendinous cylinders in sheep, an animal abundantly provided with functional muscle spindles. It is also noteworthy that musculotendinous cylinders are found in the internal or global layer of the EOM at the distal and proximal musculotendinous regions, whereas the muscle spindles of the sheep are located in the orbital layer.

1.4 PHYSIOLOGY OF EOM PROPRIOCEPTORS

The first studies on the physiology of EOM stretch receptors were by Cooper, Daniel and Whitteridge (1951, 1955). They studied responses to eye muscle stretch in barbiturate anaesthetised sheep and goats using a muscle puller that allowed them to measure tension and apply stretch simultaneously. Responses were recorded from a slip of the nerve to the inferior oblique (innervated by the oculomotor nerve) and units with resting discharges of 10 to 100 impulses/sec were recorded. The responses to EOM stretch and electrically induced twitch of these units were very similar to those seen from muscle spindles in the limb muscles. The units had a low threshold for stretch, a high rate of discharge during the rising phase of a stretch (up to 300 impulses/sec) and a slowing or pausing as the stretch was taken off. The units responded to a just maximal stimulus of the main body of the nerve to the inferior oblique with silencing of the unit during the twitch and then a burst of impulses during the relaxation period. The inferior oblique was prodded with a glass rod to identify (by peak discharge so induced) the site of the unit from which they were recording and this position was marked with methylene blue. Groups of muscle spindles were always found at these marked locations.

Whitteridge (1955) performed further experiments on the decerebrate goat. He recorded from the small nerve slips that leave the trochlear nerve and join the ophthalmic branch of the trigeminal nerve and showed that these branches do carry proprioceptive afferent information from the muscle spindles of the superior oblique. Whitteridge also recorded responses from tendon organs which fired a burst of impulses during twitch of the muscle. This result was also found by Cooper and Daniel (1957) who studied the effect of intact motor innervation on the afferent discharges from muscle spindles and tendon organs in the EOM. They concluded that removing motor input converted the afferent output from an irregular pattern with a high rate of discharge to a completely regular one, but with a much lower rate of discharge. Whitteridge (1959) isolated the γ-efferents from the cut fibres of the trochlear nerve and showed that tetanic stimulation of these efferents produced a marked increase in the static sensitivity of the spindle to stretch with a smaller increase in the "dynamic" sensitivity as well. These results led to the conclusion that the spindles in the EOM of artiodactyl ungulates were very similar, if not identical, to those found in the skeletal musculature, particularly to the widely studied muscle spindles of the cat hind-limb (Matthews, 1972).

At the time Cooper, Daniel and Whitteridge were studying the responses of EOM muscle spindles, the responses of primary and secondary spindle afferents had

not been distinguished. Although typical primary and secondary spindle endings have been morphologically identified (Harker, 1972), Browne (1975) failed to identify two populations of spindle afferent in his studies in sheep. The distribution of conduction velocities in the nerves to the superior oblique and superior rectus muscles was unimodal (30-110 m/sec) and the responses to ramp stretches, longitudinal vibration and injection of succinylcholine could not be divided into groups. However, Bach-y-Rita and Lennerstrand (1974) used similar tests to show that two such populations did exist in spindle afferent fibres in the mini-pig. Bach-y-Rita and Lennerstrand recorded their responses in the trigeminal ganglion rather than from the muscle nerves. Muscle spindle responses have also been recorded by Manni et al (1966, 1967 & 1968) from the trigeminal ganglion in lambs.

The afferent responses to EOM stretch in animals whose EOM do not contain muscle spindles have also been investigated. The majority of studies have been performed on the barbiturate anaesthetised cat. The earliest of these was by Cooper and Fillenz (1955), who recorded from slips of the nerve to the inferior oblique whilst a ligature attached to the insertion of the inferior oblique muscle was pulled. Two types of response were found. One, a low threshold receptor with either a regular, spontaneous, or sporadic resting discharge rate responded with a sharp increase in firing after EOM stretch (maximum rate 330 impulses/sec) and a cessation of activity following release of the stretch. This type of response was very similar to that seen in the sheep and goat. The second type of response was described as high threshold, the units rarely firing spontaneously and responding to stretch after a latency of 50-250 msec. This second type of receptor was associated with tendon organs. The authors comment: "There was not that feeling of exquisite sensitivity associated with the first type of response." The responses of these receptors to twitch were not investigated.

Bach-y-Rita and Murata (1964) recorded responses from small slips of the Abducens nerve in the cat whilst pulling the lateral rectus muscle. Whilst recording from the slip they were able to stimulate the main body of the nerve. "In parallel" and "in series" receptors were thus identified, depending on their response to twitch contraction, in series receptors (e.g. tendon organs) responding to twitch with an increase in firing, whereas in parallel receptors ceased firing during the twitch and resumed on relaxation. Bach-y-Rita and Murata also described a long-latency inhibitory response of spontaneously discharging motor fibres following stretch of the lateral rectus.

A further study of responses to EOM stretch in the cat was performed by Bach-y-Rita and Ito (1966). They followed a similar procedure to that of Cooper and

Fillenz, recording impulses from the nerve to the inferior oblique muscle. Stretch was imposed on the inferior oblique muscle by loading with various weights. Three different types of response were recorded. In each of four cats, a single spontaneously active receptor was found which responded to stretch with a very low threshold and a high firing rate following muscle stretch (230 impulses/sec is the highest rate shown, though no maximum rate is given). The relationship between the load applied to the muscle and the firing rate was linear for loads up to 5 grams, but increased sharply for loads above 10 grams, leading the authors to conclude that these receptors were responding to changes in muscle length for loads up to 5g and to changes in muscle tension above 10g. The responses of these four receptors were very similar to those found by Cooper and Fillenz (1955). In the remaining twenty six cats two types of non-spontaneously active receptors were found. A continuous response could be recorded from slowly adapting receptors after loading with some weights and a phasic response from rapidly adapting receptors, the response disappearing a few seconds after loading, even with the maximal weight. Bach-y-Rita and Ito comment that the spontaneously active receptors were found in experiments in which they found a large number of different units responding to stretch. Thus the presence of these spontaneously active low threshold receptors may be dependent on the state of the inferior oblique muscle and nerve. Bach-y-Rita and Ito measured the conduction velocities and dynamic and static indices of the receptors they recorded from. They found that the conduction velocities ranged from 6.5 to 52.0 m/sec. with the peak lying between 10 and 15 m/sec. The dynamic and static indices for all three types of receptor were similar; both increasing markedly with increasing initial length, suggestive of a receptor in contact with extrafusal muscle fibres rather than a region of reduced viscosity such as the equatorial region of a muscle spindle. The response of all but two of the receptors to electrical "twitch" of the muscle was a reduction or silencing of the receptor, suggestive of an "in parallel" receptor. The remaining two receptors were excited by "twitch" of the muscle and were classified as "in series" receptors, though the authors comment that "no tendon organ responses were observed." The position of receptors was located in the muscle by prodding with a glass rod, the site of the receptor being identified by a burst of impulses with each tap of the rod. All the receptors were located in the muscle belly, with the majority of low threshold receptors being located in the portion between nerve entry and globe insertion end. Bach-y-Rita and Ito concluded that "all the receptors studied appeared to be similar, suggesting that a single type of stretch receptor is located in the inferior oblique muscle of cats."

The conduction velocities recorded by Bach-y-Rita and Ito were consistently lower than those recorded by Browne (1975) in the sheep and by Batini et al (1975) in the ophthalmic branch of the trigeminal nerve in the cat (30-45 m/sec.). The diameter of degenerated fibres seen in the intracranial portions of the oculomotor nerves upon section of the trigeminal nerve reported by Buisseret-Delmas (1976) agrees with the conduction velocities recorded by Batini et al (1975), thus suggesting that Bach-y-Rita and Ito may have recorded from some fibres that did not pass through the trigeminal ganglion, and that these fibres had lower conduction velocities (Batini, 1979). Batini (1979) comments that the preterminal segments of palisade endings are 2-4μm in diameter while those of free spiral endings are 4-6μm, and adds that if these fibre diameters are maintained then the conduction velocity of free spiral endings will be higher than those of palisade endings. So it is possible that afferents from free spiral endings pass into the trigeminal ganglion, while those from palisades do not. Certainly the fact that fewer fibres are seen to degenerate following section of the ophthalmic branch of the trigeminal nerve than the number of palisade endings found in individual EOMs is suggestive that not all the afferent fibres pass through the trigeminal ganglion.

Alvarado-Mallart et al (1975) recorded responses to stretch of the cat lateral rectus muscle in the mesencephalic nucleus of the trigeminal nerve. Short latency responses were recorded, very similar both to those seen in records from the cat (Cooper and Fillenz, 1955 and Bach-y-Rita and Ito, 1969) and from recordings in artiodactyl ungulates. The units were spontaneously active with an increase in firing rate during the dynamic phase of stretch and a reduction, often ceasing to fire altogether, upon release of stretch. No detailed analysis was performed on the units to determine whether the responses were similar to those recorded from muscle spindles or like those seen by Bach-y-Rita and Ito in the nerve to the inferior oblique. Only 10 responses out of more than 200 recorded in the mesencephalic nucleus were attributed to stretch of the lateral rectus alone. To avoid the criticism that the responses were due to indirect stimulation of masticatory muscles, they tested the response of units to mechanical and electrical stimulation of the muscle. Only units that responded to both stimuli and did not respond to movements of the jaw were treated as EOM receptors. In a few experiments the nerve to the lateral rectus was severed at its distal end and electrically stimulated. Short latency responses (2 msec.) were taken as evidence that there was a direct connection from muscle nerve to mesencephalic nucleus.

Cooper and Fillenz also recorded from stretch receptors in the inferior oblique of the mangabey monkey. They comment that the muscle was extremely

small and only two responses to stretch were recorded. Neither response was from a spontaneously active receptor. One receptor responded to stretch in a similar manner to the low threshold, spontaneously active receptors they had studied in the cat; the other was similar to the high threshold receptors they had ascribed to tendon organ responses. Ito and Bach-y-Rita (1969) studied responses to stretch of the inferior oblique in the squirrel monkey. While spontaneous discharges appeared in 10 of 12 preparations, there was no increase in discharge during dynamic or static stretching of the muscle. The spontaneous activity was also abolished by administration of adrenaline, which the authors suggest is evidence that these receptors were autonomic blood vessel receptors, and they comment that some of their previous results may also be due to responses from similar receptors in the cat EOM. This is surprising since, unlike their results in the squirrel monkey, the responses seen in the nerve to the inferior oblique in the cat were modified by stretch.

Daunicht (1983) recorded responses to eye muscle stretch in the ipsilateral ophthalmic sub-division of the trigeminal ganglion in the rat. The response of these cells to increased stretch frequency was studied and it was found that a ten-fold increase of frequency produced an increased amplitude of response of only a factor of 2.2. The behaviour of these stretch receptors was summarized as intermediate between position and velocity-dependence.

1.5 AFFERENT PATHWAY FROM EOM TO BRAINSTEM

The innervation of the stretch receptors in the EOM has also been the source of much fierce discussion. Sherrington's early degeneration experiments suggested that the oculomotor (III, IV and VI) nerves were mixed afferent-efferent. Similar studies were carried out by Manni and co-workers (1968, 1970 & 1971) in lambs and pigs, animals whose EOM contain large numbers of 'classical' muscle spindles. Their results were somewhat contrary to those of Sherrington. Section of the oculomotor nerves produced degeneration of the motor innervation of the EOM, but muscle spindles and their sensory innervation remained intact, whereas section of the ophthalmic branch of the trigeminal nerve (V) had no effect on the motor innervation but produced complete degeneration of muscle spindles and their innervation. Section of the ophthalmic branch also produced degeneration in a few large fibres in the III nerve (oculomotor) and in the mesencephalon. Whilst Sherrington did see a few degenerated fibres following section of the ophthalmic branch of the trigeminal nerve (V) in the Rhesus monkey, his results are very different from those reported by Manni, et al. One explanation for this difference might be that the Rhesus monkey has far fewer muscle spindles than either lambs or pigs whereas it has a large number of musculotendinous cylinders. It may be that the two types of proprioceptors are innervated via different pathways. Buisseret-Delmas (1976) performed a number of degeneration experiments in cats and found that after sectioning the ophthalmic branch of the trigeminal nerve up to 10% of fibres were degenerated in the intracranial portion of the oculomotor (III) and trochlear (IV) nerves and 6% of fibres were degenerated in the abducens (VI) nerve .

A number of observations in a wide variety of vertebrates, with and without muscle spindles, suggest that at least some afferent information passes from initially mixed oculomotor nerves to the ophthalmic branch of the trigeminal nerve. Stibbe (1930) described small branches running from all 6 EOM to the ophthalmic branch of the trigeminal within the cavernous sinus in man. Winckler (1936, 1937, quoted in Whitteridge, 1955) found "a system of small nerve trunks running from the extraocular muscles of the artiodactyl ungulates direct to the fifth nerve." Cooper, Daniel and Whitteridge (1955) quote Kiss (1935) describing such connections in antelope, and Winckler describing these connections in pig, sheep, goat and cow. They also describe connections between the trochlear (IV) nerve and the ophthalmic branch of the trigeminal in the baboon and cat. Prince (1964) dissected the rabbit orbit and describes connections between all three oculomotor nerves and the

ophthalmic branch of the trigeminal nerve, though he comments that these branches were only found in a small number of dissections.

That these small nerve trunks are afferent nerve fibres leaving the oculomotor nerves for the trigeminal was confirmed by Whitteridge (1955) in his experiments on decerebrate goats, in which he dissected the orbit and area around the cavernous sinus and recorded responses to eye muscle stretch directly from the small branches. The responses were very similar to those seen in recordings from afferent nerves following muscle stretch in skeletal muscles. No recordings have been made in animals that do not possess muscle spindles in their EOM. That afferent signals reach the Gasserian or trigeminal ganglion has been shown by a number of studies. Manni showed responses to eye muscle stretch in the Gasserian Ganglion of lambs and pigs that were "short latency, sustained responses of the type induced by muscle spindles". However, no consistent responses were found in ten cats that were also studied.

The studies of Cooper, Daniel and Whitteridge (1953a, b) on goats included using tungsten microelectrodes to record short and long latency responses from the brainstem. Similar 'electroanatomy' was performed by Fillenz (1955) in the cat. Both studies suffer from the same criticism, that eye muscle stretch in a non-bony orbit is liable to stimulate nearby jaw proprioceptors. Responses to eye muscle stretch were found in and near the mesencephalic nucleus of the trigeminal nerve in both cat and goat, although only one cell with characteristics of a first order (primary) afferent neurone was found in the mesencephalic nucleus of the goat. In the mesencephalic nucleus of the cat Fillenz (1955) recorded from presumed primary afferent neurones with stretch of only one of the six EOM. A number of these, however, were similar to responses produced by stretching jaw muscles. Fillenz also cut the III (oculomotor) nerve and still found units responding to stretch of EOM innervated by the III nerve, thus providing further evidence that the III nerve does not carry all afferent fibres from the EOM it innervates efferently. In both studies short latency responses, characteristic of primary afferent neurones, were recorded elsewhere in the brainstem, for example, Cooper, Daniel and Whitteridge (1953a) found units within the intramedullary fibres and just caudal to the entering fibres of the trigeminal nerve.

New anatomical tracing techniques, using horseradish peroxidase as a retrograde tracer with wheatgerm agglutinin conjugated to it as an anterograde tracer allowed further study of the path of the afferent fibres from the EOM centrally. However the results have not been totally clear cut. Studies have shown the majority

of primary afferent cell bodies are located within the ophthalmic division of the trigeminal (Gasserian) ganglion in cat (Buisseret-Delmas and Buisseret, 1990, Ogasawara, 1987 and Porter and Donaldson, 1991), monkey (Porter, 1986), pigmented rabbit, (Kashii et al, 1989) and rat (Daunicht, 1985). A few tracing studies in the cat have placed a few (<25) cell bodies in the mesencephalic nucleus (Buisseret-Delmas and Buisseret, 1990 and Alvarado-Mallart et al, 1975). Porter and Donaldson (1991) suggested that these cells were a result of tracer spread from the poorly separated cat orbit to nearby jaw muscles. Buisseret-Delmas and Buisseret (1990) countered this argument by cutting the maxillary and mandibular branches of the trigeminal nerve, effectively stopping transport of tracer that had spread to jaw muscles, and still found cells containing tracer within the mesencephalic nucleus.

The distribution of primary afferent terminals within the brainstem has also caused much discussion. In the cat, Porter and Donaldson (1991) described labelling in a restricted region of the ventral portion of the pars interpolaris of the spinal trigeminal nucleus, commenting that the more caudal pars caudalis contained light labelling in cases where there was evidence of tracer spread (labelling of trigeminal motor neurones and some afferent cells in the mesencephalic nucleus). Buisseret-Delmas and Buisseret (1990) reported terminal labelling in the pars interpolaris and pars caudalis, as well as in the paratrigeminal nucleus and the dorsal horn of the cervical spinal cord. In double labelling experiments they injected the cervical dorsal horn and the EOM, and found double labelled cells in the trigeminal ganglion. Ogasawara et al (1987) found terminals predominantly in the rostral pars oralis of the spinal trigeminal nucleus and moderate labelling in the principal trigeminal nucleus. One reason for the observed differences may be that slightly different protocols were used in the three sets of experiments outlined above. Porter and Donaldson injected a relatively large volume of WGA-HRP (35 µl) into two or three EOM using minimal dissection of the orbit to expose the EOM, whereas Buisseret-Delmas and Buisseret injected a much smaller volume of WGA-HRP (1µl) into all six EOM, which may well have involved a larger amount of blunt dissection to expose the EOM, as would the sectioning of the maxillary and mandibular branches of the trigeminal nerve near the ganglion. Ogasawara used a relatively small volume of WGA-HRP (5µl) and injected into multiple EOM, but gives no further details as to the amount of dissection used to expose the EOM. Porter (1986) studied the afferent pathway in macaque monkeys and found labelled terminals in a restricted portion of the ventro-lateral pars interpolaris and in the pars triangularis of the cuneate nucleus, partially overlapping the area reached by dorsal neck muscle afferents. This second projection was not found in any of the studies on projections of EOM afferents in the

cat. One unusual finding was that of Eden and co-workers (1982) who found labelled cell bodies in the spinal trigeminal nucleus of the pigeon, but did not look in the trigeminal ganglion for cell bodies. Eden et al used unconjugated HRP at a very high concentration which may well have spread to other structures, particularly since exposing the EOM will have involved considerable dissection (see Chapter Four for a discussion of these surprising results).

It therefore appears that at least some primary afferent cell bodies are located in the trigeminal ganglion, with terminals in the ipsilateral trigeminal spinal nucleus, although the exact location remains unclear. However, there is also evidence that some afferent fibres travel in the "motor" nerves. Tarkhan (1933) confirmed Sherrington's degeneration experiments for the oculomotor and trochlear nerves and comments that the number of fibres in the trochlear nerve doubles after passing through the region of the mesencephalic trigeminal nucleus, which he suggests may be the source of proprioceptive fibres. Woolard (1931) cut the oculomotor nerve and found degeneration of all nerve fibres in the nerves to EOM innervated by the oculomotor nerve, and degeneration in the Oculomotor nucleus and a restricted portion of the mesencephalic trigeminal nucleus. Corbin and Harrison (1940) recorded from the mesencephalic trigeminal nucleus and found no responses to EOM nerve stimulation. Corbin and Oliver (1942) lesioned the mesencephalic trigeminal nucleus and found no degenerating fibres in any of the oculomotor nerves. They also lesioned the oculomotor and trochlear nuclei and saw no degeneration in the mesencephalic nucleus, but did see degeneration of motor nerve endings and "insertion third grape-like endings" in the EOM. Cody and co-workers (1972) recorded in the mesencephalic nucleus and found no responses to eye muscle stretch. Bach-y-Rita and Murata (1964) recorded from the root of the abducens (VI) nerve in the cat just external to the brainstem and found lateral rectus stretch receptor responses. Sas and Schab (1952 quoted in Alvarado-Mallart and Pinçon-Raymond, 1979) found degeneration of palisade endings following destruction of the oculomotor nuclei (III, IV and VI), a result consistent with the studies of Sherrington (1898, 1912). Taren (1964) lesioned the trigeminal mesencephalic nucleus and found degenerating fibres in the oculomotor and abducens nucleus. Further evidence that some afferent information may reach the brainstem via the abducens nerve comes from two studies by Batini and co-workers (1974, 1975); in the first, Purkinje cell responses upon stimulation of the peripheral stumps of the EOM motor nerves were abolished in the case of the oculomotor and trochlear nerves following section of the ophthalmic branch of the trigeminal nerve, whereas the responses seen following stimulation of the abducens (VI) nerve were diminished but not removed. The

second experiment recorded from the trigeminal ganglion following electrical stimulation of peripheral oculomotor nerve fibres, and responses were found after stimulation of all three oculomotor nerves. Section of the ophthalmic branch of the trigeminal nerve produced a few (3-5%) degenerated fibres (30-100 fibres/nerve) in the peripheral branches of the oculomotor nerves, and section of the oculomotor nerves did not cause degeneration of all myelinated nerves in the same.

1.6 PATHWAY OF EOM PROPRIOCEPTION BEYOND THE FIRST SYNAPSE

For many years the study of Cooper, Daniel and Whitteridge (1953a, b) of central responses to EOM stretch was the major source of information about the central pathways of the EOM stretch receptor afferents. As well as first order responses to EOM stretch already mentioned above, responses with longer latencies, suggestive of second order or later neurones were found in the medial longitudinal fasciculus, central tegmental tract, reticular formation, superior colliculus, occipital lobe, cerebellar tracts and cerebellum. These results have all been confirmed and extended by a number of later studies.

Electroanatomy using either electrical stimulation or stretch of individual EOM in the cat has shown responses in lobules V-VII of the cerebellar vermis (Fuchs and Kornhuber, 1969; Baker et al, 1972 and Schwarz and Tomlinson, 1977); in the deep (Fillenz, 1955 and Batini and Horcholle-Bossavit, 1977), intermediate (Abrahams and Rose, 1975 and Rose and Abrahams, 1975) and superficial (Donaldson and Long, 1977, 1980) layers of the superior colliculus; in the lateral geniculate and contiguous nuclei (Donaldson and Dixon, 1980; Lal and Friedlander, 1989, 1990a, 1990b); in the visual cortex (Buisseret and Maffei, 1977; Enomoto et al 1983 and Ashton et al, 1984-passive eye movement was used to stimulate EOM receptors) and the Clare Bishop Area (Donaldson, 1979); in the reticular formation (Fillenz, 1955); in the medial vestibular nucleus, nucleus praepositus hypoglossi and related brainstem structures (Ashton et al, 1988a-passive eye movement was used to stimulate EOM receptors); in the oculomotor nucleus (Tomlinson and Schwarz, 1977) and in neck and forelimb muscles (Easton, 1971a, 1971b, 1972 and Manni et al, 1975).

Whilst the cat has been the animal in which most studies on the projections of EOM afferents have been carried out, there have also been studies in a number of other species. Azzena et al (1970) showed responses to EOM stretch with similar latencies to those found in the cat (10-50 msec) in the cerebellar vermis in lambs. In the goldfish Hermann (1971) recorded saccadic evoked potentials in the optic tectum (TSEPs) which were affected by EOM stretch. Dissection of the EOM away from the globe greatly reduced the ipsilateral TSEP and increased the movements of the contralateral eye. Responses to passive eye movement in the giant toad (*Bufo marinus*) were found in the vestibular nuclei (Ashton et al, 1984b) and in the vestibular nuclei, cerebellum and oculomotor nucleus of the rainbow trout (*Salmo gairdneri*) (Ashton et al, 1989). In the pigeon (*Columba livia*) Donaldson and Knox

(1990a, 1990b, 1991) have shown responses to passive eye movement in the vestibular nuclear complex, the oculomotor and abducens nuclei and the reticular formation. Interactions with visual stimuli have also been noted in the pigeon tectum (Dr. P.C.Knox, personal communication). Hayman et al (1993a, 1993b) have shown responses to imposed eye movement on the electromyographic (emg) activity of neck muscles of the pigeon, and Manni et al (1975) have shown responses to EOM stretch in the emg of neck muscles in lambs. Maekawa and Kimura (1980) have shown a mossy fibre projection of EOM afferent signals to the rabbit cerebellar flocculus.

1.7 FUNCTIONAL PURPOSE OF EOM PROPRIOCEPTION "PURELY REFLEX TAXIS OF LOCAL POSTURE AND MOVEMENT"

The oculomotor system is one of the most studied areas of motor control, and according to Huber (1988) is said to be one of the best understood neuromuscular systems. While there have been numerous reviews of eye movements and their control (Whitteridge, 1960; Carpenter, 1988; Robinson, 1981) none of the recent reviews has given any role to proprioceptive signals from the EOM. Indeed Robinson almost echoes Carpenter's comments, (see Introduction) saying "So little is known about the signals carried by these pathways, however, that there is very little support for any of the several hypotheses that have been advanced to explain the function of stretch afferents in eye muscles".

Fuchs and Kornhuber (1969) suggested that "EOM stretch receptors play a role in a cerebellum-mediated proprioceptive feedback loop for the control of eye movements, providing information to the cerebellum as to the magnitude and the end point of saccades." Baker et al. (1972) suggested that "the cerebellar cortex may, therefore, be involved in the 'correction' of movement prior to its execution, and this predictive ballistic type of correction may be continuously updated by the presence of feedback from the extraocular muscle receptors." Schwarz and Tomlinson (1977) investigated responses to EOM stretch of the cat cerebellar vermis in lobule VI whilst the muscles were still attached to the globe and found responses that were plane-specific and specific to one particular direction within that plane. They also described one pyramidal cell that was excited by stretch of the left lateral rectus and inhibited by stretch of the right lateral rectus.

While the cerebellar vermis does play some role in eye movement, the cerebellar flocculus is known to integrate various sensory signals including visual and vestibular signals, and to be involved in the control of eye movements (Ito et al, 1982 and Lisberger, 1988). The findings of Maekawa and Kimura (1980) and Kimura and co-workers (1980, 1981) that floccular Purkinje cells responded to particular parameters of passive eye movement, specifically eye velocity and position and the direction of eye movement, is evidence that functionally significant extraocular muscle afferent signals are available to the flocculus. That these signals do play a role in the control of eye movements was shown by Kimura et al (1991) who sectioned the ophthalmic branch of the trigeminal nerve and observed "changes in the movement dynamics of the ipsilateral eye", producing a decrease in high frequency vestibuloocular reflex (VOR) gain. They showed that this effect was due to pathways coursing through the flocculus by lesioning the flocculus with injections

of kainic acid. This produced very similar effects on the VOR gain to those seen following section of the ophthalmic branch of the trigeminal nerve and the effects were not increased by subsequent section of the ophthalmic branch.

EOM proprioception and the VOR

The canal vestibuloocular reflex (VOR) is a relatively simple mechanism for stabilising the retinal image during head movement. Vestibular stimulation can be applied simply via a vestibular turntable onto which an experimental animal can be placed. It thus provides a simple eye movement reflex open to investigation. EOM proprioception is generally thought not to play a role in the control of the VOR. Oculomotor modellers (Robinson, 1981 and Guitton, 1988, 1992) have produced models of the VOR which solve the various integrations and transformations of the angular acceleration applied to the head (and sensed by the vestibular system) and output an eye movement signal that matches the physiological reality with some precision. Notwithstanding the prevailing opinion, the fact that EOM proprioceptors might play some part in the VOR has been suggested by a number of workers.

Gernandt (1968) showed a marked inhibition of vestibular activity in reticular neurones following EOM stretch or oculomotor nerve stimulation, but no effect was seen on neurones within the classical three neurone arc of the VOR, i.e. in the medial longitudinal fasciculus or oculomotor nuclei (the vestibular nuclei were not investigated). It is interesting to note that facilitatory responses to EOM stretch or nerve stimulation were never observed, only inhibitory ones. Gernandt explained his results by stating that it appeared that proprioceptive signals from the EOM only modified vestibulo-ocular impulses conducted through the brainstem reticular formation. The results were gained in anaesthetised cats, following a cerebellectomy, which may have removed some of the effects of the EOM proprioceptors on the VOR (see Carpenter, 1972, Kimura et al, 1991).

Allum and Graf (1977) proposed a model of the VOR that included an EOM proprioceptive signal feeding back onto the vestibular nuclei to increase the time constant of vestibular neurones during vestibular nystagmus. This model was supported by the findings that the time constant of vestibular nuclei neurones was no different from that of vestibular afferents in goldfish that had been paralysed, but significantly lower than that seen in vestibular nuclei neurones in goldfish that were free to move their eyes. Allum and Graf ascribed this result to a proprioceptive feedback loop from the EOM, because a feedforward loop from the vestibular nuclei neurones would produce effects seen only at a later stage in the VOR. They also felt

that the result could not be explained by a corollary discharge signal, because this would not have been affected by paralysis, which would also be true of any feedforward mechanisms.

The continuing research of Donaldson and co-workers into the effects of EOM proprioception on the VOR was in part stimulated by Allum and Graf's work. As already mentioned, Donaldson's group has shown the effects of EOM afferent signals on various brainstem areas involved in the VOR in a number of different species with different repertoires of eye movements. More recently, Donaldson and Knox (1991, 1993) have studied the effects of imposing an 'artificial VOR' on various stages of the VOR, including its output. Briefly, slow, sinusoidal movements are imposed on one eye using a suction contact lens. Thus the eye can be moved at the same speed, but in the opposite direction to the head movement, i.e. imposing a compensatory VOR on the eye. Increasing or decreasing the velocity of the eye movement imposes systematic errors on the VOR, producing eye movements that are too fast or too slow for the imposed vestibular stimulation.

Using this technique Donaldson and Knox have shown that neurones in the oculomotor nuclei, the electromyogram of individual EOM and movements of the whole globe all show systematic changes in their vestibular response which can be explained by a 'corrective' functional hypothesis. Thus movements of the eye that are slower than a compensatory VOR produce an increase in the response, be it the number of impulses in the oculomotor nuclei, the gain of the electromyographic activity in an EOM, or movement of the globe, as measured by the electrooculogram. Similarly, movements of the eye that are faster than would compensate for the imposed head movement (i.e faster than the compensatory VOR) reduce the gain of the response. These results suggest that EOM afferent signals do reach centres involved in the VOR and produce corrections of the reflex from moment to moment.

The results of Donaldson and Knox are corroborated by those of Kashii et al (1989) and Fiorentini and Maffei (1977). Kashii et al showed that the slow phase of the VOR is disrupted when the ophthalmic branch of the trigeminal nerve is cut in the rabbit; similarly, Fiorentini and Maffei cut the ophthalmic branch in the cat and observed slow pendular movements of the eye on the operated side in the dark, and an altered movement response to vestibular nystagmus. Whilst these two studies show that EOM proprioceptive signals do have an effect on the VOR, they add no additional information to Donaldson and Knox's finding that the effects of EOM afferent signals affect the VOR from 'moment to moment'.

Both Kashii et al and Fiorentini and Maffei's studies were made some days after the surgery. The effects on the VOR could, therefore, be due to parametric, or

long term calibration effects of deafferentation; such an effect was first proposed by Ludvigh (1952a). Parametric feedback is the use of long term error signals, not to affect a movement in progress, but to improve further performance. Steinbach (1987) quotes evidence of a slow recalibration of spatial localization in patients having undergone enucleation of one eye. Such slow changes have also been reported in patients with sudden onset paralysis of the eye muscles (Leigh and Zee, 1983), and in the adaptation to changes of saccadic amplitudes following unilateral tenectomy of the medial and lateral rectus muscles in the monkey. Lewis, Zee and Guthrie (1992) studied the effect of deafferentation on disconjugate adaptation in monkeys with unilateral vertical muscle weakness and concluded that "proprioceptive information is used in the long term calibration of the internal efferent copy map of the position of the eyes in the orbit." Carpenter (1977) strongly champions the 'cause' of long-term parametric adjustment in the first edition of his book, 'Movements of the Eyes', quoting, amongst others, experiments in which pairs of extraocular muscles are transposed (Leinfelder and Black, 1941). Within a matter of days there was a recovery of cooperation between the EOM. Olmstead et al (1936) found a similar correction following an EOM pair transposition in a blind eye. Surprisingly this section did not appear in the revised edition of the book (Carpenter, 1988).

EOM stretch reflex

The presence of a stretch reflex in the EOM has been a subject of controversy since Sherrington reported evidence of responses to stretch of the inferior oblique muscle in cats and macaque monkey (1893). McCouch and Adler (1932) found no evidence of a stretch reflex upon pulling EOM in an enucleated cat and quote Hoffman (1929) as reporting no EOM stretch reflex, though the species studied is not mentioned. One of the most elegant studies on reflex actions of EOM was performed by Keller and Robinson (1971) in alert, behaving rhesus macaque monkeys. They used an opaque suction contact lens to impose movements on one eye whilst the monkeys were fixating with the other eye. Recording from the ipsilateral Abducens nucleus, no effect was found on the activity of Abducens motorneurones. Similarly, clamping the eye during a conjugate saccade to produce an isometric saccade had no effect on single-unit or background multi-unit activity. Keller and Robinson concluded that "the monkey was shown unequivocally not to have an extraocular muscle stretch reflex" during periods of constant motor innervation (fixation) or changes in the central motor commands (saccadic eye movements). They further

stated that "no extraocular muscle, or other orbital receptor, plays a role in reflex or short-term alteration of motorneurone discharge rate through any afferent feedback mechanism." Keller and Robinson do, however, comment that "the monosynaptic stretch reflex in skeletal muscle is only one, low order, spinal function driven by stretch afferents." They in no way ruled out a role for EOM proprioception in more global proprioceptive functions via projections to the cerebellum.

Early literature on the stretch reflex in Man is conflicting, various authors stating the presence of a tonic reflex (Breinin, 1957), an inhibitory reflex (Sears et al, 1959) or marked stretch reflex (Maruo, 1964). Recently Tamura and Mitsui (1986) have provided interesting data on a long latency reflex they describe as the "magician's forceps phenomenon" in which passive deviation of the non-deviating eye in exotropic strabismics produces temporary alignment of the deviating eye. The effect has been shown under general anaesthesia and in EMG records in alert patients. Inter-ocular proprioception has also been shown in the cat under general anaesthesia (O'Keefe and Berkeley, 1991).

Studies in the cat have also shown long latency reflex effects of EOM stretch. Bach-y-Rita and Murata (1964) reported a long latency inhibitory stretch reflex on spontaneously active Abducens motorneurones following stretch of the lateral rectus, but suggested that this may be an inhibitory reflex of the retractor bulbi muscle. Bach-y-Rita (1972) also reported an inhibitory stretch reflex produced only when efferent activity was present in fibres of the Abducens nerve. A similar inhibitory response (hyperpolarisation) in oculomotor motorneurones was shown by Sasaki (1963). Taylor (1965) studied the activity in oculomotor neurones before and after curarisation of the EOM during vestibular stimulation. He states that since there is no change in the phase of the response, EOM proprioception cannot be involved in the short term control of vestibular eye movements. However, close examination of his figures shows a reduction in gain of approximately 20%, in other words an inhibition of the response.

Tomlinson and Schwarz (1977) reported responses of oculomotor motorneurones to EOM stretch in the ventral portion of the oculomotor nucleus. Long latency (34-170 msec) excitatory and inhibitory responses were recorded following stretch of individual ipsilateral and contralateral EOM. Some of the units in the oculomotor nucleus were identified as motorneurones by stimulation of the nerve to the ipsilateral medial rectus muscle producing antidromic spikes. The conduction velocity of these motorneurones was slow (30-50 m/sec). This finding is interesting because Keller and Robinson (1971) quote Baichenko and co-workers (1968), who hypothesised that the EOM stretch reflex was mediated solely by the

slow muscle fibres in the EOM, and make the point that their study does not rule out this hypothesis because it is likely that such motorneurones may have been missed in their study due to the preponderance of fast twitch motorneurones.

The studies of Donaldson and Knox in the pigeon (1990a, 1991, 1993) have shown predominantly inhibitory effects of passive eye movement on neurones located in the abducens and oculomotor nuclei during vestibular stimulation. While motorneurones were not positively identified in these nuclei, all units were affected by passive eye movement, so it would be unlikely that only slowly conducting motorneurones (i.e. innervating slow muscle fibres) were recorded from. It is noteworthy that Keller and Robinson did not study the effect of imposed eye movements during vestibular stimulation. It is possible that the reflex effects of eye movement only appear "in context". As Keller and Robinson noted, EOM proprioception may play a role in eye movements via projections to the cerebellum. As noted earlier, the cerebellum is intimately involved in the control of the VOR. Gernandt (1968) did not find any effect of eye muscle stretch in the oculomotor nuclei; however he was using cerebellectomised cats. Kimura and co-workers (1991) showed that disruption of the VOR following floccullectomy was not increased by section of the ophthalmic branch of the trigeminal nerve.

Keller and Robinson's finding that no reflex effects are seen during saccadic eye movements is confirmed by the study of Guthrie, Porter and Sparks (1983) who cut the ophthalmic branch of the trigeminal nerve and showed that perturbations of eye position produced by microstimulation of the superior colliculus were still compensated for, presumably by a corollary discharge signal. Such signals are known to reach the superficial layers of the superior colliculus (Richmond and Wurtz, 1977).

While muscle spindles found in the EOM of Man are somewhat 'non-standard' (Ruskell, 1979); those in the macaque sparse (Greene and Jampel, 1963) and absent in the cat and pigeon (Maier et al, 1974), the muscle spindles found in the artiodactyl branch of the ungulata are very similar histologically and physiologically to those found in the skeletal musculature. The absence of a stretch reflex in the EOM of the goat (Whitteridge, 1962) suggests that the stretch reflex is indeed absent from the EOM.

The absence of a 'classical' monosynaptic stretch reflex in the EOM is not, however, a strong piece of evidence against a role of EOM proprioceptive signals in the short term control of eye movements. The archetypal stretch reflex described by Liddell and Sherrington (1924, 1925) was studied in a hind-limb extensor muscle and the reflex action was referred to the joint, producing the brief 'tendon jerk' reflex

known to clinicians. The EOM do not contain functional extensors, flexors or joints, thus comparisons between such differing muscle systems are, at the least, tenuous, if not meaningless. Granit (1971) comments that "The statements to the effect that stretch reflexes are absent in the extrinsic eye muscles need not be taken too seriously.", adding that stretch reflexes are absent in the skeletal musculature of normal subjects under normal conditions. He also quotes evidence from Collins et al (1971) that the tension-extension curves of an extrinsic eye muscle are parallel at whatever angle of gaze stretching is begun, suggesting that this is strong evidence of spindle load compensation. Carpenter (1988) refutes such a conclusion with evidence from electrical stimulation of EOM nerves in the cat which showed that the mechanical properties of the EOM affect the length-extension curves in a manner identical to that seen by Collins et al, and therefore, the observed results provide no evidence of spindle involvement in load compensation.

The absence of a stretch reflex does beg the question, what are the muscle spindles seen in EOM there for? One interesting idea put forward by Steinbach and co-workers (1990, 1992, Harris et al, 1993) is that spindles are present in the EOM of animals in which the centre of rotation of their eyeball is not co-incident with the centre of gravity. In such animals the spindles would be present to correct the posture of the eyes, a sort-of anti-gravity function. In a similar, if more involved sense, Cooper and Daniel (1949) propose that the presence of muscle spindles in even-toed ungulates is due to these animals having a high centre of gravity and thus, "The remarkable balance of goats and deer may well be aided by impulses from the eye muscles."

1.8 FUNCTIONAL PROPERTIES OF EOM PROPRIOCEPTORS "ELABORATION OF VISUAL SPACE"

Muscular feeling and muscular sense

Location of an object in space relative to the body requires summation of three signals:

i) the position of the image on the retina.

ii) the position of the eye in the orbit relative to the head.

iii) the position of the head relative to the body.

While the sources of signals i) and iii) are generally considered to be understood (Jeannerod, 1983), the source of the signal representing eye position within the orbit remains unclear.

Debate over an eye position, or extra-retinal, signal has continued for well over a hundred years. The progenitors of the debate are two of the most eminent scientists of their generations, Hermann von Helmholtz and Sir Charles Sherrington. Traditionally, Helmholtz is cited as being the source of the idea that internally generated signals might have perceptual consequences, though others had expressed similar ideas earlier (see McCloskey, 1981). In Helmholtz's 'Treatise on Physiological Optics' (1929) he defined as *muscular feeling:*

"The sensations which enable us to perceive changes of position of the parts of the body through muscular action." He divided the term into "several essentially different sensations", distinguishing them:

"1. The *intensity of the effort of will*, whereby we endeavour to bring the muscles in action;

2. The *tension of the muscles*, that is, the force by which they try to act; and

3. The *result of the effort*, which, regardless of its being perceived by other organs of sense, such as sight and touch, makes itself felt in the muscle by a contraction which actually takes place, and in which it may be possible to perceive after a fashion the change of tension of the skin over the parts affected."

Helmholtz presents two experiments to show that in the case of eye movements it is the *intensity of the effort of will* that plays the major role in any muscular feeling. Using a modification of the eye-press illusion originally described by Descartes (1664), he pulled on the skin at the side of a viewing eye with the other eye occluded, producing apparent motion of objects in the field of view. The positions of after images, however, appeared to stay where they were when the eye

was so pulled. Helmholtz argued that movements of the eye produced in such a manner caused changes in the length of the extra-ocular muscles (EOM) without muscular action, and thus "our judgement as to the direction of the visual axis is not formed either by the actual position of the eyeball or by the actual elongation or contraction of the ocular muscles that is the result of this position."

That the tension of the EOM was not involved in the judgement of visual direction was, Helmholtz believed, shown by experiments with patients, some of whose EOM were either wholly or partially paralysed. Helmholtz observed that in patients with a wholly paralysed EOM, as long as eye movements were not attempted in a direction that required action of the paralysed EOM, the directions of objects were correctly observed in the field of view. However, when the patient attempted to deviate the eye towards the paralysed EOM, the eye remained in a central position while the objects appeared to move, yet the position of the eye and the retinal images within it had not changed. In a patient with a partially paralysed EOM, the impaired muscle requires a greater degree of innervation than needed under normal conditions, and an incorrect idea of the visual axis is gained which produces mislocations in reaching for objects.

Thus Helmholtz concluded that "These phenomena prove conclusively that our judgements as to the direction of the visual axis are simply the result of the effort of will involved in trying to alter the adjustment of the eyes", and in his Review of the Theories (p.533) that, "We feel also the degree of innervation which we cause to be communicated to the nerves of the ocular muscles."

Sir Charles Sherrington vehemently disagreed with the theory of "sensation of innervation" as he called Helmholtz's views. His own experimental work on spinal reflexes led him to develop his definition of the *muscular sense*, a notably similar term to Helmholtz's muscular feeling. Sherrington's definition of muscular feeling is, however, very different:

"all *reactions on sense arising in motor organs and their accessories.*"

Sherrington's arguments for muscular sense within the EOM were often anecdotal or observations rather than rigorous experiments; however many of his comments have since been shown to have considerable basis in fact. Perhaps his experiments in the periphery where he gained the early evidence for a muscular sense led him to believe that a similar sense might be present in the EOM. Sherrington countered Helmholtz's paralysed eye experiment by quoting James, who believed that the effects might be due to movements of the occluded eye being a source of peripheral sensation. Using examples predominantly of paralysed limbs, Sherrington concluded that "sensation of innervation - remains unproven".

In his 1918 paper on EOM proprioception Sherrington does demonstrate an extremely simple experiment involving Listing's Law to show that a signal other than the position of images on the retina is necessary for the elaboration of visual space, and claims ocular proprioceptors as the source of this additional signal. He does not mention 'sensation of innervation' at all, although this 'sense' would provide an equally suitable signal.

Does EOM proprioception provide a sense of eye position?

The absence of a sense of eye position has been used as a strong argument against a role for EOM proprioception in eye movement control. It was first reported by Irvine and Ludvigh (1936), who grasped the anaesthetised surface of the eye with forceps and found the subject was unable to state the position of his eye and had no sensation that his eye had moved. Brindley and Merton (1960) anaesthetised the conjunctiva with cocaine and occluded the eye with an opaque aluminium shell. Movements of the occluded eye produced by grasping the insertion of the medial or lateral rectus with forceps thrust through the conjunctiva were not detected, nor were movements of, or changes in visual perception in the contralateral eye noticed. Similarly, movements of both eyes by up to 30° (to obviate James' (1907) objections to studies on a single eye ignoring the orientation of the unaffected eye) were undetected.

Skavenski (1972) performed similar experiments using a suction contact lens to impose movements on one eye with the other occluded with an eye patch. Using this technique and a forced choice paradigm known to be more sensitive than the subjective assessment used in earlier studies, the two trained subjects in Skavenski's study were able to report correctly the presence of a load on the eye and the direction of the pull on a highly significant (>80%) proportion of trials ($p<0.001$) with eye movements no greater than 10°. Similar results were gained in an experiment of Ludvigh's (1952b) in which subjects had to predict the direction in which they were looking at an image following random image displacement a few degrees to the left or right. Subjects were able to tell which way they were looking on more than 75% of occasions with deviations of 6° or more in the horizontal plane and 3° or more in the vertical plane. Ludvigh believed that this represented very poor positional sense compared to that seen in limbs or the accuracy with which visual movements are registered by the retina. He further commented that the better performance in the vertical plane may well be due to cues gained from the mechanoreceptors in the

eyelids. Skavenski (1972) performed a second experiment with his contact lens equipment which provides more convincing proof of an accurate eye position signal. The same subjects viewed a visual target which was extinguished and the lens subsequently loaded with weights that would produce a passive displacement of up to 5° whilst the subject was instructed to maintain their eye position. This was achieved with surprising accuracy, even with relatively small loads that would have displaced the eye by only a few degrees (2.5°).

The role of EOM proprioception in visual perception

Helmholtz's experimental evidence for his theory of sensation of innervation has long been regarded as near proof that EOM proprioception plays no part in spatial localization. However, recent evidence has done much to 'dent' this view. The fall from grace of Helmholtz's views has been excellently documented by McCloskey (1981). Helmholtz's observations on the eye-press illusion have been the source of considerable interest from other workers. The basic illusion that pressing on the outer canthus of one eye produces illusory movement of the visual field has been confirmed many times (Jackson and Paton, 1909; Irvine and Ludvigh, 1936; Skavenski et al, 1972; Bridgeman and Stark, 1991). Bridgeman and Stark (1991) have shown that a sustained press on the outer canthus of a fixating eye produces a change in the innervation of the eye so that it opposes the eye press leaving eye position constant. The same form of eye press does produce rotation in a non-viewing eye. That constant position will produce a constant afferent signal from the EOM has also been advanced by Skavenski et al (1972) who studied visual localization whilst applying loads to a scleral contact lens applied by suction to a fixating eye. In both the above mentioned studies the perceived direction of a visual target (while studied using different psychophysical techniques) was altered in a direction close to that which would have been produced by the motor innervation present in the EOM of the fixating eye if that eye were free to move. Both sets of authors considered their results as proof that a copy of the motor innervation (efference copy or corollary discharge signal) was used to judge the location of objects in space. Skavenski et al (1972) did not consider that proprioceptive signals from the EOM would have been produced by the changed motor innervation because the position of the eye was constant. This is very unlikely, however, because recordings from EOM proprioceptors have shown that they respond to tension as well as position (Bach-y-Rita and Ito, 1966). Bridgeman and Stark (1991) comment that tendon organ receptors responding to tension might have been responsible for

some of the visual mislocalization they observed, but they note that mislocalizations do not occur during saccades when such receptors would produce a large signal due to the large tensions produced in the EOM muscles. This explanation ignores the fact that Houk and Henneman (1967) have shown that tension receptors (Golgi Tendon organs) in the cat hind-limb are stimulated by contraction of a small number of muscle fibres in series with the receptor, and that contraction of muscle fibres not in series with the receptor can produce 'unloading' (inhibition) of the receptor. In EOM the musculotendinous cylinder is the main proprioceptor found in the tendinous regions of EOM. These endings are closely associated with a single, slowly contracting, multiply innervated muscle fibre. If, as is probable (Ruskell, 1979), the palisade ending responds to muscle contraction in a similar manner to Golgi tendon organs, it is quite possible that saccadic eye movements produce unloading of these receptors, since such eye movements are believed to be produced predominantly by singly innervated muscle fibres. The slower contractions seen in the eye-press experiments of Bridgeman and Stark (1991) may well stimulate the musculotendinous receptors, producing afferent signals affecting visual localization.

That no movements of the visual field are observed when movements are imposed on an unseeing eye has been claimed by a number of workers (Brindley and Merton, 1960; Skavenski et al, 1972). Skavenski et al (1972) also showed that perceived visual direction is unaltered by imposed movements of a non-seeing eye via weights applied to a scleral contact lens. Recent results do not, however, support this finding. Gauthier et al (1990a, b) used a similar procedure and found subjects' perception of visual direction as tested by open loop pointing to a visual target was deviated in the direction of the passive eye movement. Gauthier et al (1990a) comment that their results, while appearing to contradict those of Skavenski et al, fall within the bounds of the error predicted between pure outflow and the actual results. Their protocol, requiring localization of a single target, is also simpler than that used by Skavenski et al whose experiment involved two targets. Using a different method to produce ocular misalignment (sustained eye-press on a non-viewing eye) Bridgeman and Stark (1991) showed visual mislocalizations of a similar magnitude and in the same direction, towards the eye deviation. Gauthier's study was prompted by his earlier findings of visual mislocalizations in strabismic patients (Mandelbrojt et al, 1986). In 65% of patients studied, visual mislocalizations were seen in the direction of the non-viewing eye whether the normal or strabismic eye was used for fixation.

A recent paper by Lewis and Zee (1993) has provided further strong evidence of a role of EOM proprioceptors in the perception of visual direction. Using a patient

with a congenital trigeminal-oculomotor synkinesis, an unusual condition in which the patient's left medial rectus was abnormally co-activated with the left lateral pterygoid muscle, perception of visual direction was tested monocularly with the right eye by a similar method to that of Gauthier et al, either with the jaw deviated to the right, to stimulate the left lateral pterygoid and left medial rectus muscles, or the jaw straight ahead. The effect of rightward jaw deviation was a marked adduction (nasal movement) of the left eye (~42°) and mislocation of the target in the opposite direction to the eye movement. This result appears contradictory to the studies by Gauthier et al and Bridgeman and Stark on the effects of passive eye deviation which found mislocations in the same direction as passive movement. Lewis and Zee explain this discrepancy by illustrating the difference in the activation of musculotendinous cylinders produced by passive adduction of an eye compared to synkinetic adduction. In passive adduction of an eye, musculotendinous cylinders in the lateral rectus will increase in activity (stretch of the lateral rectus increasing the tension at the tendinous margins), whereas those in the medial rectus will decrease in activity (as muscle length is shortened, so reducing tension). In synkinetic adduction, musculotendinous cylinders will still increase in activity in the lateral rectus (for the same reason), but will also increase in activity in the medial rectus (since with active contraction of the muscle, tension will increase). They also commented that receptors signalling muscle length, such as muscle spindles would produce identical signals (an increase of firing by receptors in the lateral rectus and a decrease of firing in the medial rectus) during passive or synkinetic adduction. Lewis and Zee, therefore, concluded that afferent signals from musculotendinous cylinders signalling muscle tension were responsible for the 'elaboration of visual space' rather than signals from receptors, including muscle spindles, that signal muscle length.

The startling illusory movements of the visual world that patients with paralysis or weakening of one or more EOM experience upon attempting a saccadic eye movement in the direction of the muscle paralysis or weakening has been well documented (Helmholtz, 1929; Mach, 1875; Jackson and Paton, 1909; Cogan, 1956). Later workers recreated these illusions using partial paralysis of the EOM with local anaesthetic or paralysing drugs such as curare (Kornmüller, 1931; Brindley and Merton, 1960; Stevens, 1976; Matin, 1982) or by preventing movement of the eye (Mach, 1975) and showed that in addition to illusory jumps of the visual world, subjects mislocated visual targets in the direction of the attempted eye movement. However Siebeck (1954) reported that completely curarised subjects perceived no illusions of spatial displacement. This result directly contradicts the theory of sensation of innervation put forward by Helmholtz (1929) and championed by many

other workers (e.g. Merton, 1964). Complete paralysis of the EOM has been repeated by two groups (Brindley et al, 1976 and Stevens et al, 1976). Brindley et al completely paralysed one eye with retrobulbar injection of local anaesthetic and found no illusory jumps or target mislocalizations (past pointing errors), even when "it is only those muscles actually needed for the attempted movement that are completely paralysed." Additionally Brindley et al investigated movements of retinal after images, which they believed would strengthen the case for a proprioceptive role in the control of eye movements. They hypothesised that if proprioceptive signals were responsible for eye position information, an after image in the non paralysed eye should move with the eye during a saccade, but such an image in the paralysed eye should remain stationary. They reported that one subject "clearly found just this", while their second subject thought it "almost certainly true for him."

Stevens et al (1976) studied partial paralysis of the EOM with increasing doses of curare and complete paralysis, either by systemic injections of a neuromuscular blocking agent (succinylcholine) or retrobulbar injection of local anaesthetic. They reported four distinct perceptions after partial paralysis: "1. *displacement* or repositioning of the perceived visual world in the direction of a successfully directed eye movement; 2. *jumping* during a saccade; 3. *movement* associated with drift of the eye and 4. increased *effort* associated with each eye movement." After complete paralysis with succinylcholine, jumping during an intended saccade disappeared, but the other perceptions seen under partial paralysis remained. Complete paralysis with retrobulbar injection of local anaesthetic removed all perceptions, save one of displacement, and consequent past pointing disappeared. Stevens et al believed that their results were explained by the uncovering of three different systems. The sense of effort was ascribed by them to EOM proprioceptors, because this sense was abolished by retrobulbar block but was still present in paralysis with succinylcholine. They stated that the local anaesthetic block effectively abolished putative EOM proprioceptors, whereas the receptors were intact under paralysis with succinylcholine. Jumping and movement (drift) were ascribed to a pattern visual system, and displacement, which was described as "non-visual", and past pointing to a corollary discharge signal.

Considerable emphasis (McCloskey, 1981) has been placed on Stevens' subjective reports under succinylcholine paralysis that a strong sense of effort was required to move the eyes and on his large past-pointing errors that were in the direction of the intended movement, as evidence that corollary discharge signals do play some part in visual stability. These perceptions were never experienced in the study by the Brindley et al (1976). One possible reason for the discrepancy between

the two studies is that succinylcholine causes strong contraction of the slow, multiply innervated muscle fibres found in the EOM, rather than neuromuscular block. The musculotendinous cylinder is closely associated with individual multiply innervated muscle fibres, and is likely to be preferentially stimulated by them. Thus Stevens' sense of effort and mislocalizations may well be due to strong afferent firing from musculotendinous cylinders. This hypothesis does not, however, explain the mislocalizations experienced following retrobulbar block. Brindley et al do comment on the difficulty of obtaining complete paralysis of the eye by this method. Stevens et al (1976) were unable to use their scleral contact lens system to measure eye position during the retrobulbar block experiments due to orbital oedema; it is possible that incomplete paralysis is the explanation to the two studies' conflicting results.

The importance of the complete EOM paralysis experiments is indicated by the comment from McCloskey (1981): "The observations made during complete oculomotor paralysis remove the strongest piece of evidence supporting a role for corollary discharges in visual stability and instead provide compelling evidence against such a role." Carpenter (1988) counters: "it seems that some retinal image movement is a prerequisite for the sensation of movement of the visual world: but if this retinal slip can be accounted for by a concomitant eye movement, it is ignored. The observations with completely paralysed eyes are therefore not as devastating for the outflow theory as they perhaps seem at first, although they rule out a simple model in which perceived external movement is under all circumstances equal to the difference between retinal image movement and desired eye movement."

Studies of visual localization in patients with EOM proprioceptive deficits provide further evidence of a role of EOM proprioception in visual localization. Steinbach and Smith (1981) studied visual localization in patients undergoing their first or subsequent operations for surgical correction of strabismus. Open loop pointing tasks in patients who had been operated on for the first time and whose eyes had been covered after the operation until the moment of testing, showed errors in spatial localization with the surgically rotated eye that were no more than 25% of the rotation. Small errors were also noted in localizations made with the unaltered eye. Steinbach and Smith explained this result by stating that eye position information from EOM proprioceptors must have been available to correct for the imposed surgical eye rotation, given the absence of any visual information. In addition to this, Steinbach and Smith showed that patients undergoing surgery for the second or more time did have large visual mislocalizations with the operated eye, but only slight mislocalizations with the unaltered eye. This appears to be in agreement with an

outflow theory, whereby eye position information is gained from a copy of the motor command sent to the eyes. Steinbach and Smith argued that this result was due to slowly developing scar tissue caused by the successive surgeries deafferenting the EOM by removing proprioceptive sense organs from the tendinous insertion. Evidence in support of this theory came from Richmond et al's (1984) study of the site of strabismus surgery and the presence of musculotendinous cylinders within this region. Steinbach et al (1987) later showed that patients undergoing marginal myotomy surgery for strabismus, in which the musculotendinous insertion region of the EOM is first crushed before half cuts are made on opposite sides of the muscle, showed much greater visual mislocalizations than patients who had undergone recessions, in which the tendinous insertion is moved without disrupting the musculotendinous portion to any great extent. The marginal myotomy surgery would obviously have a far greater detrimental effect on musculotendinous cylinders than the recession procedure, which suggests that musculotendinous cylinders are indeed involved in the mislocalizations seen after strabismus surgery.

Bock and Kommerel (1986) repeated Steinbach and Smith's experiments but did not find a similar separation of results between patients undergoing their first operation and subsequent operations, all patients showing spatial mislocalizations of a similar order to the surgical rotation of the eye. Steinbach (1987) comments that this may have been due to differences in surgical technique; Bock and Kommerel used retrobulbar injection of local anaesthetic, whereas Steinbach and Smith used general anaesthesia in all their operations. Gauthier et al's (1987) results on strabismic patients may shed some light on this phenomenom. They studied strabismic patients' subjective straight-ahead direction and visual mislocalizations using open-loop pointing tasks similar to both Bock and Kommerel and Steinbach and Smith's studies. Their preliminary results suggest at least two types of strabismic patient. One in which the strabismus is due to "an essentially mechanical factor affecting the resting position of the eye". Mislocalizations are therefore made when viewing with the strabismic eye (see also Mandelbrojt et al, 1986) pre-operatively which are corrected by the surgery and secondly, where the strabismus is due to "inappropriate nervous activation affecting permanently, and more or less constantly one muscle". Pre-operatively the patient has little visual mislocalization viewing with either eye, but post-operatively there are large mislocalizations when the operated eye is used. Gauthier et al comment that their classification is not exhaustive and there may therefore be other types of deficit producing further permutations pre- and post-operatively. Furthermore the study of Salvi et al (1989) showing that the musculotendinous cylinders of strabismus patients are abnormally

innervated suggests that EOM proprioceptive signals from patients with strabismus may be different to those from normal subjects. Studies on the effects of strabismus surgery on visual localization are thus likely to be harder to interpret than the explanations suggested to date.

Further compelling evidence that visual localization is at least in part due to proprioceptive signals from the EOM comes from the research of Campos et al (1986). They studied patients with herpes zoster ophthalmicus. In this unusual condition the ophthalmic branch of the trigeminal nerve is infiltrated by a virus effectively deafferenting the structures innervated by this nerve. While it is not known for certain that proprioceptive signals travel in this nerve in humans, studies in primates and other species strongly suggest that this is the case. Campos and co-workers found large visual mislocalizations in the affected eye of patients during the active phase of the infection that disappeared with remission. This last study strongly suggests not only that EOM proprioceptive signals affect visual localization, but that these signals enter the brain via the ophthalmic branch of the trigeminal nerve in Man as they are known to do in a wide variety of other species.

The role of EOM proprioception in development

A large number of animal studies have provided further convincing proof of a role for EOM proprioceptive signals in the development and normal function of visual behaviour. As mentioned earlier, responses to EOM stretch or electrical stimulation of the intraorbital portions of oculomotor nerves have been seen in the visual cortex and dorsal lateral geniculate and associated nuclei (Buisseret and Maffei, 1977; Donaldson and Dixon, 1980; Enomoto et al, 1983; Lal and Friedlander, 1989). Maffei (1979) and Berardi et al (1981) found that following unilateral section of the ophthalmic branch of the trigeminal nerve in kittens 5-6 weeks old, there was a decrease in the number of binocularly activated cells in the striate cortex, 60% of cells responding only to monocular stimulation. Recently, Trotter et al (1991, 1993) have shown a low level of binocularity and disparity sensitivity in adult cats that had undergone unilateral section of the ophthalmic branch of the trigeminal nerve during a critical postnatal period (see later). This permanent loss in binocularity was ascribed to a reduction of binocular suppression and to a selective increase in the variability of the binocular response.

Buisseret and co-workers have conducted an excellent and exhaustive series of experiments into the role of EOM proprioception in the development of orientation selectivity in visual cortical neurones (see Buisseret, 1979, 1992 for

reviews). Initially they showed that in 6 week-old dark reared kittens there were virtually no orientation specific cells (Imbert and Buisseret, 1975), but with only 6 hours of visual experience orientation selectivity was restored in most neurones. Thus visual experience was shown to be necessary for the development of orientation selectivity. That "extraretinal" factors might also be involved in this development was suggested by the work of Held and Hein (1963) and by the fact that no orientation selectivity was found to develop in dark reared kittens during recording sessions that lasted in excess of six hours or in paralysed kittens with normal vision and passive movements to simulate normal visual experience (Buisseret et al, 1978). To test the type of self-directed movements that were necessary to develop orientation selectivity, kittens were immobilized in plaster of Paris so that only their eyes were free to move (Buisseret et al, 1978). Visual exposure under these conditions led to full development of orientation selectivity. Conversely, kittens with unrestricted body movement, but surgical immobilization of the eyes either by removal of all the EOM or section of the oculomotor nerves, showed no recovery of orientation selectivity (Gary-Bobo et al, 1986).

That proprioceptive signals from the EOM were necessary for the development of orientation selectivity in kittens was shown by the almost total lack of development of orientation selective neurones following unilateral section of the ophthalmic branch of the trigeminal nerve in 6 week-old dark reared kittens with 6 hours of visual experience using self-directed eye movements (Buisseret and Gary-Bobo, 1979). Bilateral section of the ophthalmic branch also prevented development of orientation selectivity in the majority of visual cortical neurones following visual experience for 6 hours in 6 week-old dark reared kittens (Trotter et al, 1981b). Following 4 weeks of normal visual exposure, a slow and incomplete recovery of orientation selectivity was noted. This has not been confirmed for kittens with unilateral section of the ophthalmic branch of the trigeminal nerve. Bilateral section of the maxillary branch of the trigeminal nerve did not stop development of orientation selectivity after only six hours of visual exposure, further suggesting that it is the proprioceptive information from the EOM that is required for this development. Buisseret and Singer (1983) also showed that bilateral section of the ophthalmic branch of the trigeminal nerve reduced vision dependent modifications of cat visual cortical neurones produced by monocular deprivation or surgically induced strabismus.

That proprioceptive information about eye position is necessary for the development of visually guided behaviour has been shown by a series of studies by Hein and co-workers (see Hein and Diamond, 1983 for a review). Thus the

development of visually guided locomotion and reaching has been shown to require self-directed eye movement and intact proprioception from the EOM. Hein et al also showed that once visually guided behaviour had been acquired, EOM proprioceptive information was not required to maintain this behaviour, suggesting that EOM proprioception is required only for initial calibration of eye movements and eye position. However, they also showed that visually guided behaviour in the absence of proprioceptive information required continual visual exposure to prevent degradation of the behaviour; no such degradation was observed in normal kittens following similar periods in the dark. EOM proprioceptive information is, therefore, probably still used after the acquisition of visually guided behaviour to provide eye position information.

Studies by Graves et al (1987) and Trotter et al (1991) have also shown that EOM proprioception plays a role in the development of depth perception in kittens. Unilateral or bilateral deafferentation of the EOM by section of the ophthalmic branch of the trigeminal nerve produced deficits in binocular depth perception within certain temporal limits. Depth perception was tested using a modification of the method devised by Mitchell et al (1979) - the jumping stand technique. Briefly cats had to jump from a starting platform to a landing surface directly below. The cats were trained to jump onto either the left or right side of the platform depending on the depth of two similar patterns composed of random dots illuminated from below. The height of the right and left surfaces was varied; a correct choice was the nearer surface. Kittens with either unilateral or bilateral EOM deafferentation showed deficits in depth perception compared to controls (e.g. sham operated kittens). Trotter et al (1991) showed that the temporal limits of this effect started at 3 weeks for both unilateral and bilateral deafferentation and ended at around 11 weeks for bilateral deafferentation and 14 weeks for unilateral deafferentation. Trotter et al comment that the earlier upper limit seen with bilateral deafferentation may be due to complete removal of an EOM proprioceptive signal blocking, or freezing the normal development of visual functions, an effect described by other workers as the 'gating' role of EOM proprioceptors (Buisseret and Singer, 1983; Trotter et al, 1981a). At around 10 weeks binocular depth thresholds have reached their adult levels in normal kittens; therefore, blocking visual development will have no effect on binocular discrimination. Conversely, unilateral deafferentation does not block synaptic plasticity, and subsequent visual development, rather the imbalance of EOM proprioceptive signals during a period in which layer 6 visual cortical neurones are still susceptible to unilateral deafferentation (Trotter et al, 1987) may well cause the observed effects on binocular depth perception. That the deafferentation procedure

does not have a purely visual or visuomotor action was shown by the fact that binocular depth perception was not disrupted until four days after the operation to section the ophthalmic branch of the trigeminal nerve.

Whilst the above experiments provide compelling evidence that EOM proprioceptive signals are involved in the development of both visual cell properties and behaviours in the kitten, they suggest that after a critical period of development, proprioceptive deafferentation is unlikely to affect the adult cat. The work of Fiorentini and Maffei and co-workers on the adult cat, however, does not fit with this theory. Starting from their finding of instability of the cat eye in the dark (Fiorentini and Maffei, 1977, see earlier) following unilateral deafferentation by section of the ophthalmic branch of the trigeminal nerve and the reduction in binocularly-activated cells in the visual cortex of adult cats following surgical paralysis of one eye (Maffei and Fiorentini, 1976), errors in orienting behaviour and depth discrimination following unilateral EOM deafferentation in the adult cat have been found (Fiorentini et al 1982, 1985, 1986).

That unilateral deafferentation produces systematic errors in depth discrimination in the adult cat is in direct conflict with the work of Graves et al (1987) who found no such errors in deafferented adult cats. Trotter et al (1993) comment that the differences "may be due to the variation of magnitude and often to the lack of vergence movements to near objects in cats (Hughes, 1972)". It has, indeed, been shown that following section of the ophthalmic branch of the trigeminal nerve in monkeys, normal vergence movements and maintained convergence during fixation are impaired (Guthrie et al, 1982). Fiorentini et al (1985, 1986) were aware of this criticism of the 'jumping stand technique' and studied depth discrimination using an operant conditioning technique with a periodic stimulus (grating) rotated in depth. Such a technique should, the authors comment, "minimize the possible effects on stereoacuity of an incorrect alignment of the eyes" (Fiorentini et al, 1986). A further possible reason for the discrepancy between the studies of Graves et al (1987) and Fiorentini et al (1985, 1986) are the observation made by Fiorentini et al (1985, 1986) that new behavioural strategies, such as pendular head movements producing motion parallax, were developed by the cats which improved both monocular and binocular depth discrimination. It is possible that the cats used in Graves et al's (1987) study had gained behavioural strategies pre-operatively which might have affected their post-operative performance.

The pathways by which EOM proprioceptive information reaches the visual cortex have also been studied. Lal and Friedlander (1989) showed that EOM stretch affects visual signals in the dorsal lateral geniculate nucleus (dLGN) and Donaldson

and Dixon (1980) recorded impulses following EOM stretch in the perigeniculate region which passes signals to the dLGN. Guido et al (1988) reported a shift in the proportion of X and Y cells in the dLGN following surgical immobilization of one eye, the balance being restored by section of the ophthalmic branch of the trigeminal nerve of the unparalysed but not the paralysed eye. Both layers 4 and 6 of the striate cortex receive geniculate afferents, and layer 6 neurones project back to the dLGN. Trotter et al (1993) suggest that "during postnatal development EMP [extraocular muscle proprioception] signals would exert an efferent control of dLGN transmission through the corticogeniculate loop to allow the congruency between retinal mapping and eye position mapping to construct a precise and reliable network for spatial interocular disparity coding at the cortical level." The results of Guido et al (1988) were on adult cats suggesting that similar geniculo-cortico-geniculate control might be responsible for the effects of eye immobilization and EOM deafferentation seen in adult cats (Fiorentini et al, 1982, 1985, 1986).

1.9 CONCLUSIONS

This review has presented evidence for a number of different types of EOM stretch receptor, including muscle spindles, musculotendinous cylinders and free nerve endings. The distribution and structure of muscle spindles appears to suggest that they have little proprioceptive function in species other that the artiodactyl ungulates. The musculotendinous cylinder has been identified in all species lacking muscle spindles, and these receptors fulfil the anatomical requirements of a proprioceptor. However, while responses to single eye muscle stretch have been recorded from the oculomotor nerves and the trigeminal ganglion, no recordings from identified musculotendinous cylinders have been achieved. The importance of the musculotendinous cylinder as opposed to free nerve endings or as yet unidentified proprioceptors is therefore uncertain.

The afferent fibre pathway from EOM proprioceptor to the brainstem has, in common with many aspects of EOM proprioception, been a subject of considerable debate. Most researchers would now accept that the vast majority of first-order afferent neurone cell bodies are located within the ophthalmic subdivision of the trigeminal ganglion. The possibility that a few cell bodies may also be present within the trigeminal mesencephalic nucleus in the cat is made uncertain by problems with tracer spread to nearby jaw muscles. Afferent terminals from these first-order neurones are generally accepted to lie within the spinal trigeminal nucleus, centred in the pars interpolaris subdivision of this nucleus.

That afferent signals elicited by EOM stretch or passive eye movement do reach many areas of the brain involved in the control of eye movements and vision is now without doubt. The problem has been to ascribe a functional purpose to these 'electroanatomical' findings, as noted by Carpenter (1988) (see section 1.1). Deafferentation of the EOM by section of the ophthalmic branch of the trigeminal nerve in cat and rabbit has been shown to produce instability of the eye and disruption of the slow phase of the VOR, suggesting that an important control signal has been removed. These studies are complemented by those of Donaldson's group which have shown that EOM afferent signals induced by passive and imposed eye movements are involved in the moment-to-moment control of the VOR in the pigeon.

Interesting results from both animal studies and Man have shown a considerable role of proprioceptive signals from the EOM in the development of visual cortical cell properties and in egocentric localization. Thus proprioceptive deafferentation of the EOM produces behavioural deficits in binocular depth

discrimination tasks and visuo-motor behaviour which have been correlated with physiological effects in the visual cortex such as reductions in the number of binocularly activated cells in the visual cortex and their orientation selectivity in the kitten. Similar results have also been reported in the adult cat. In a number of studies on Man, subjects with various forms of altered proprioceptive signals from their EOM have been shown to produce visual mislocalizations in open-loop pointing tasks, providing strong evidence that EOM proprioceptive signals are intimately involved in the elaboration of visual space.

In the skeletal muscle system views on the role of proprioceptive signals and corollary discharges have recently converged such that most researchers now believe both signals are necessary in the generation of position sense, corollary discharges providing a template upon which to decode inflowing afferent signals (Matthews, 1982; McCloskey, 1981). Such hybrid signals have also been proposed for the oculomotor system (Shebilske, 1977; Matin, 1976) and, perhaps, this is the most parsimonious explanation of our present understanding of this system. That afferent signals from the EOM do play some part in the control of eye movements and vision must now, however, be certain.

CHAPTER 2. EFFECTS OF IMPOSED EYE MOVEMENTS ON THE VESTIBULOCOLLIC REFLEX OF THE PIGEON DURING VESTIBULAR STIMULATION.

2.1 INTRODUCTION

The stabilization of gaze (eye and head position) in space is an essential requirement for various tasks such as visuo-motor co-ordination, spatially-oriented behaviour and visual tracking. Head movements generate a bilateral pattern of vestibular afferent activity in the semicircular canals and macular receptors of the inner ear. Within this activity the direction and velocity of head displacement is encoded. Vestibular afferent activity elicits reflexes in the eyes and head as well as postural adjustments in the axial musculature.

The vestibuloocular reflex (VOR) helps stabilize the retinal image during head rotations by producing rotations of the eyes that are nearly equal and opposite to head rotations. The vestibulocollic reflex (VCR) gives rise to reflex excitation of the appropriate neck muscles to resist and counteract head displacement. The reflex output of the VCR (head stabilization) modifies the canal input and, therefore the reflex error signal. The VCR thus comprises a closed-loop control system which serves to stabilize the head on the neck. The VCR can be studied as an open-loop reflex by fixing the head and recording neck muscle electromyographic (EMG) activity or head torque (Bilotto et al, 1982). The VOR, on the other hand, is an open-loop control system in which the input (head movement) is unaffected by the output (eye movement).

A number of other reflex systems interact with the vestibular reflexes to stabilize the head and eyes. Optokinetic reflexes (OKRs), evoked by movements of the visual field in relation to the animal, allow gaze stabilization relative to visual surroundings. The OKRs generate adequate stabilization of the head and eyes at low frequencies, with the vestibular reflexes providing most of the gaze stabilization at frequencies above 0.5 Hertz (Gioanni, 1988).

Movements of the head on the body or vice versa also elicit proprioceptive reflexes in the neck muscles. The cervico-collic reflex (CCR) potentiates the VCR if the neck muscles are being stretched during vestibular stimulation and attenuates the VCR if they are shortening (Dutia and Price, 1987). The cervico-ocular reflex (COR) produces eye movements during movements of the head on the body, but these are relatively weak (Barmack et al, 1989; Fuller, 1980).

The effect of extraocular muscle (EOM) proprioceptive reflexes produced by eye muscle stretch have not generally been considered to play a part in the reflex control of eye or head movements (e.g. Dutia, 1991). However, evidence has recently shown that EOM proprioceptive signals do indeed affect eye movements during the VOR (Knox and Donaldson, 1993).

A number of researchers have also shown that eye muscle signals do reach neck muscles during various orienting behaviours. Easton (1971a, b, 1972) showed patterned inhibition in neck and forelimb muscles resulting from rotation of the eye, or stretch of a single eye muscle in the anaesthetised cat. To summarise his findings: neck muscles received considerably more inhibition than forelimb muscles, lateral rotation produced a distinctly stronger effect than medial rotation and ipsilateral muscles received more inhibition than contralateral muscles. Control experiments strongly suggested that the source of the inhibition was from receptors located in the EOM.

Manni et al. (1975) repeated Easton's studies in the anaesthetised cat and extended their study to examine responses in lambs and rabbits. They confirmed Easton's finding of bilateral neck muscle inhibition in response to EOM stretch in the cat, found no response in the rabbit, and ipsilateral contraction and contralateral inhibition in the lamb. This pattern of neck muscle activation was also seen in lambs in response to electrical stimulation of the trigeminal ganglion or within the descending Trigeminal nucleus. However, similar responses were noted in neck muscles following pinching or rubbing of the face and electrical stimulation of other Trigeminal nerves, suggesting that the responses to EOM stretch were non-specific responses to stimulation of Trigeminal afferents.

The presence of eye position and/or eye velocity signals in second-order vestibular neurones has been shown by a number of researchers (Berthoz, et al, 1981; McCrea et al, 1981; Yoshida et al, 1981). Morphological study of these neurones showed that they projected not only to the ipsilateral or contralateral abducens nucleus, but, also, axon collaterals projected to areas of the brainstem involved in eye and head coordination (prepositus nucleus, medial reticular formation, etc.) and also to the spinal cord (McCrea et al , 1980, 1981). Eye position signals have indeed been found in brainstem areas such as the prepositus nucleus (Lopez-Barneo, 1982; Baker and Berthoz, 1975), and in dorsal neck muscles, innervated from the cervical spinal cord.

Berthoz and co-workers have studied the coupling between eye and head movements during orienting gaze shifts in the horizontal plane. This coupling is very tight in lower animals (e.g. the rabbit, Fuller, 1980), although in higher animals the

ability to dissociate eye and head movements has progressively increased (Ron and Berthoz, 1991).

The coupling has been studied using chronically implanted electromyographic electrodes and ocular search coils and both physiologically and morphologically at the cellular level in great detail using the technique of intra-axonal recording in the alert cat. The main finding has been that there is a close correlation between the activity of dorsal neck muscles and the horizontal component of eye position in the orbit. In addition to a dynamic coupling, with ocular saccades synchronised with phasic bursts of neck muscle activity, a tonic coupling has been discovered. Thus, when the eyes move to the left there is an increase of electromyographic activity of left neck muscles proportional both to eye position and to abducens motor neurone discharge, while the activity in contralateral neck muscles is reduced or inhibited. Furthermore, during vestibular stimulation, the VCR can be completely suppressed or facilitated depending upon whether gaze position is directed to, respectively, the contralateral or the ipsilateral side. This coupling, both phasic and tonic, has also been found in the monkey (Lestienne et al, 1984) and in man (Andre-Deshays, 1988, 1991).

The neural origin of the signal is still uncertain. While a number of brain stem neurones appear to carry phasic, eye velocity, information to the spinal cord (tectoreticulospinal and reticulospinal neurones) and these may also contain some decremental tonic activity, Berthoz (1992) suggests second order vestibular neurones as the best candidates for a tonic eye position signal (vestibulo-oculospinal neurones) reaching the spinal cord, although an, as yet not fully characterised, type of reticulospinal neurone, dubbed "quasi-tonic" (Berthoz, 1992) also has elements of a tonic eye position signal in its firing characteristics.

The source of the tonic eye position signal is generally ascribed (Vidal et al, 1983; Berthoz, 1992) to a corollary discharge signal produced by the oculomotor neural integrator. The vestibular nuclei and the prepositus nucleus are the most probable anatomical site for this integrator. The fact that EOM proprioceptive signals do reach these nuclei (Ashton et al, 1988; Donaldson and Knox, 1990a; Donaldson and Knox, 1993) does suggest a role for such signals in the generation or correction of the corollary representation of eye position (Andre-Deshays et al, 1988; Berthoz, 1992).

The fact that afferent signals from the EOM induced by passive eye movement affect the vestibular activity of single units in the same brainstem areas believed to be the source of the eye position and velocity signals responsible for producing the striking effects of eye position on the mammalian VCR suggested that

studying the effects of imposed eye movement on the electromyographic activity of dorsal neck muscles during vestibular stimulation might prove fruitful.

The decerebrate pigeon (*Columba livia*) has been used in the experiments described hereafter. This is an excellent preparation for studying responses to imposed eye movements since it is stable and avoids the complicating effects of general anaesthetics. It has an intact afferent pathway from the EOM to brainstem areas which may be compromised in mammalian decerebrate preparations and Donaldson's group has already shown the existence of interactions between EOM afferent signals and various brainstem areas involved in the VOR in the decerebrate pigeon (Donaldson and Knox, 1990a, 1991; Knox and Donaldson, 1993). The pigeon has excellent visual acuity (Hodos et al, 1985) and makes a wide repertoire of eye movements, including saccades of up to 15° (Bloch et al, 1981), a form of vergence used in binocular feeding (Bloch et al, 1981) and a well developed VOR (Anastasio and Correia, 1988; Gioanni, 1988), making it a good model for higher vertebrates.

2.2 MATERIALS AND METHODS

2.2.1 Preparation

Pigeons (*Columbia livia*), more than ten weeks old, were anaesthetised with ether. The skin covering the skull was cut with a scalpel and manually drawn apart to reveal the skull. The skull was opened with bone rongeurs, the dura overlying the cerebral hemispheres cut and the cerebral hemispheres removed by aspiration, leaving the optic lobes, cerebellum and brainstem intact. Cotton wool covered in absorbable gelatine powder (Gelfoam powder, Upjohn, Michigan) was placed onto the circle of Willis. Direct pressure and suction on the cotton wool for approximately 30 seconds stopped any bleeding. After recovery, evidenced by the return of vestibular reflexes, the bird was placed on a servo-controlled vestibular turntable. The pigeon's head was placed into a head-holder positioned so that the semicircular canals were aligned over the axis of rotation of the turntable to prevent any appreciable tangential or centrifugal linear accelerations of the head during rotation. The head-holder was designed so that the pigeon's beak was angled down approximately 40° from the earth horizontal. In this position the horizontal semicircular canals are approximately aligned with the earth horizontal (Donaldson and Knox, 1990a). This is also the natural angle at which undisturbed pigeons hold their heads (Anastasio and Correia, 1988; Erichsen, 1989).

Exposure and section of the ophthalmic branch of the trigeminal nerve was achieved by removing bone and dura from the overlying tectum which was subsequently removed by aspiration (Zeigler et al, 1975). The dura overlying the dorsal surface of the trigeminal ganglion was removed and the ophthalmic branch which courses antero-dorsally towards the foramen orbitorotundum, was sectioned with a 10A scalpel blade. Confirmation of the section was provided by careful dissection at the end of the experiment.

2.2.2 Stimuli

Natural vestibular stimulation (VEST) was provided by sinusoidal oscillations at frequencies between 0.2 and 2.0 Hertz at amplitudes between ±3° and ±8° peak-to-peak in the horizontal plane. Usually the VEST was at 0.4 Hz with an amplitude of ±8°. The gain of the pigeon closed-loop vestibulocollic reflex (CL-VCR) is approximately constant when tested with sinusoidal oscillation at frequencies between 0.2 and 2.0 Hz (Gioanni, 1988) and appears to be independent

of stimulus magnitude. Stimulus magnitudes above 30°/s produce a slight decrease in the gain of the CL-VCR and a considerable decrease in the gain of the VOR. Therefore, the stimulus magnitude in the following experiments never exceeded 30°/s. The CL-VCR shows a small phase lead on velocity at low frequencies (0.2 Hz), which decreases as the frequency of vestibular stimulation increases (Gioanni, 1988).

EOM proprioceptive signals were induced by imposed movements of one, usually the left, eye. The eye was moved by an electromagnetic servo-controlled device that acted upon a stalk carried by an opaque contact lens held firmly to the cornea and sclera by suction. Local anaesthetic (lignocaine 1%) was routinely applied to the cornea before application of the lens and at regular intervals thereafter. The apparatus was adjusted so that the stalk was held approximately along the optical axis of the eye; all eye movements started from that position. The eye mover could be rotated about the axis of the stalk so that eye movement could be produced along any desired radius; this rotation was driven by a stepper motor controlled by the laboratory computer so that the radius along which the next eye movement would take place could be controlled by the computer program. The direction of imposed eye movement (IEM) which was produced by the computer program was adjusted by inputting the direction required in degrees, such that 0° corresponded to vertically upwards, 90° to horizontally tailwards, 180° vertically downwards and 270° horizontally towards the beak (see Figure 2.10) All rotations of the eye mover to alter the plane of IEM took place only when the stalk was in the central 'starting' position to avoid movement of the globe during such rotations.

The type of imposed eye movement (IEM) was also controlled by the computer program; there were two basic types:-

i) Trapezoidal, starting with a rapid movement at constant velocity from the central position along a particular orbital radius to a new position, which was held for a short time (usually 200 ms), before retracing its path to the centre. The three phases of this stimulus were labelled S1, S2 and S3 respectively (see Figure 2.1). The parameters of this trapezoidal stimulus were controlled by the computer program, allowing different velocities, amplitudes and hold-times to be imposed. The time during the sinusoidal, vestibular stimulus cycle at which the IEM occurred was similarly controlled. Imposed velocities ranged from 19 to 140°/s. The pigeon's normal saccadic velocity is ≈120°/s (Bloch et al, 1981). Amplitudes of IEM varied from 5° to 20°.

ii) Sinusoidal in time course, always at the same frequency as the vestibular turntable, but in the opposite direction, thus mimicking the slow phase of the VOR. With VEST at 0.4 Hz, peak table velocity was 22°/s. An imposed sinusoidal eye movement with the same peak velocity as the table and thus the head, but in the opposite direction, would produce an 'artificial VOR' (aVOR) with a gain of -1. Altering the amplitude or phase of the imposed sinusoid, via the computer program, allowed controlled errors of the aVOR to be studied (see Figures 2.1 and 2.4).

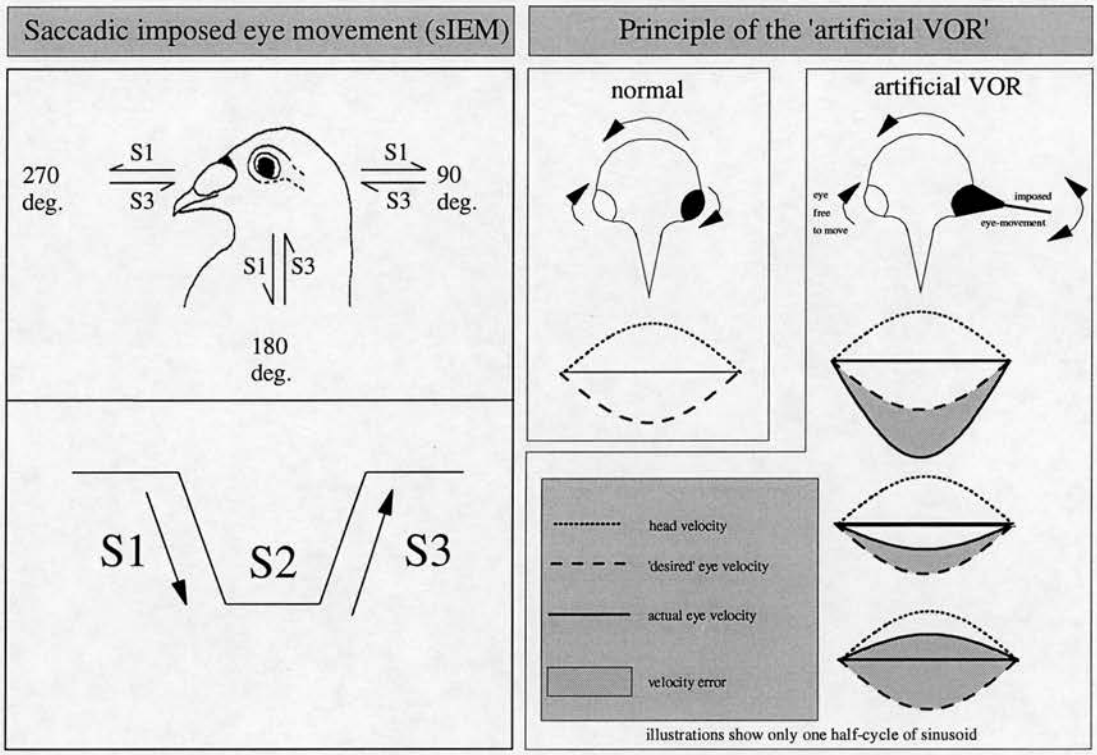

Figure 2.1. Diagrammatic illustration of the two methods of imposed eye movement used. Saccadic imposed eye movement (sIEM) (left-hand side), and the artificial VOR (right-hand side). When an experiment used sIEM the eye was held centred in the orbit and then moved rapidly (S1) to an eccentric position in a pre-set direction, three of which (beakwards, 270°; tailwards, 90° and vertically downwards 180°) are illustrated. After being held at the new position for a period of time (S2) the eye was returned to the centre of the orbit (S3). The trapezoid shown in the lower part of the Figure shows the phases of the stimulus as they appear on the eye-position analogue records in succeeding figures (adapted from Donaldson and Knox, 1990a). The illustration of the principle of the artificial VOR (aVOR) (right-hand side) illustrates the movement of the eye in response to vestibular stimulation, the normal VOR. The *artificial* VOR panel illustrates how eye movement imposed on the left eye can be adjusted to provide, from above down, excessively high eye velocity, excessively low eye velocity and eye velocity too low and also in the wrong (anti-compensatory) direction. The velocity error in each case is the *hatched area* (adapted from Donaldson and Knox, 1993). The aVOR could also be used to provide imposed eye movement with controlled phase errors (see Figure 2.23).

2.2.3 Apparatus and computer program

Overall control of the experiment was through the laboratory computer, a Tandon PCA30 personal computer. The computer program was written in Turbo Pascal (Borland) and contained a number of screen menus that were used to select and control the variables of a particular experiment (see Figures 2.2, 2.3 & 2.4). A CED 1401 (Cambridge Electronic Design Ltd., Cambridge), an interrupt-driven programmable interface connected to the laboratory computer, was used to send analogue driving signals to both the vestibular turntable and eye mover via two digital-to-analogue converters, a digital driving signal to the stepper motor used to rotate the eye mover about a horizontal axis and to sample, collect and digitise the amplified neck muscle electromyographic activity and position signals from both vestibular turntable and eye mover via three analogue-to-digital converters. Eye movement was synchronised with the vestibular stimulation by simultaneous, multiplexed driving signals from the digital-to-analogue converters of the CED 1401. This was achieved by a custom-made command (TWODAC, CED, Cambridge) downloaded from the laboratory computer to the CED 1401.

Natural vestibular stimulation (VEST) in the horizontal plane (yaw) was produced by a heavy, horizontal, servo-controlled vestibular turntable on which the bird was placed. For VEST in the frontal (roll) and sagittal (pitch) planes, the bird was placed on a platform attached to a servo-controlled direct-current printed-circuit motor (G16M4 Printed Motors Ltd, Bordon, Hants). Imposed eye movements (IEM) in the horizontal plane were produced using a servo-controlled electromagnetic eye mover bolted to the vestibular turntable and attached to a stepper motor that could rotate the eye mover through 360° controlled by the CED 1401 as described above. IEM in the frontal and sagittal planes was produced by a similar servo-controlled electromagnetic eye mover attached to the vestibular turntable. A stepper motor was not connected to the eye mover, so eye movements were only possible in one plane (the vertical plane).

2.2.4 Recording and collection of data

The four neck muscles tested were identified by dissection and with reference to an atlas of avian anatomy (Chamberlain, 1943). *Complexus* is the most superficial dorsal neck muscle in the pigeon; it originates from the transverse processes of the second, third, fourth and fifth cervical spines and has a large insertion onto the occipital crest. Its action is to extend the neck dorsally and laterally. *Rectus capitis*

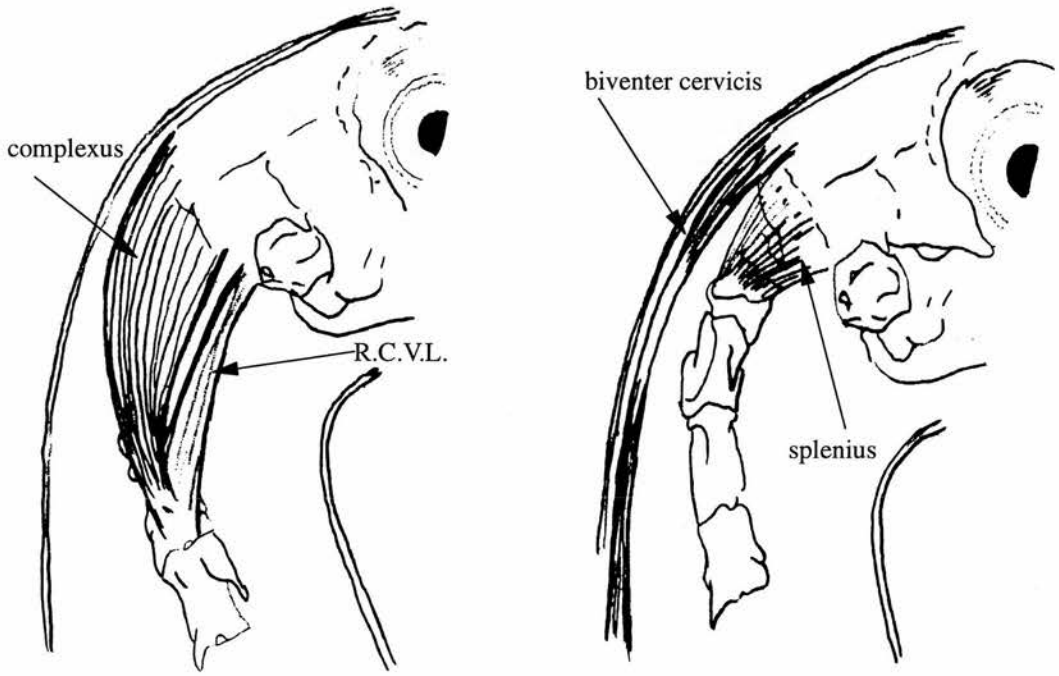

Figure 2.1a. Diagram to show the four neck muscles studied during the course of the present study. For details of the actions of these muscles see section 2.2.4.

dorsalis major lies deep to *complexus* and *biventer cervicis*. It is similar to *splenius* in most mammals (George and Berger, 1966), and will henceforth be described as *splenius*. It originates from the dorsal spines of the second, third and fourth cervical spines and inserts on the mediodorsal part of the nuchal surface of the occipital bone. Its action is to extend the head on the neck when the two muscles are acting together. When it acts on one side only the head is also turned to that side. *Biventer cervicis* lies bilaterally in the midline deep to *complexus*, its origin is from the supraspinous ligament to the first and second lumbar vertebrae and it inserts on the nuchal surface of the occipital crest. Its action is to flex the head dorsally. *Rectus capitis ventralis lateralis* lies lateral to *complexus*, originating from the transverse processes of the fourth and fifth cervical vertebrae and inserting on the nuchal surface of the occipital bone, its action being ventral and lateral flexion of the head on the neck.

Neck muscles were exposed by dissection. *Complexus* and *rectus capitis ventralis lateralis* were exposed by cutting and retracting the skin and connective tissue overlying these superficial muscles. *Splenius* and *biventer cervicis* were exposed following intramuscular injection, into the large pectoralis muscle, of pentobarbitone sodium (Sagatal, May and Baker, 3 mg diluted in 5 ml 0.9% saline solution) to sedate the bird. *Biventer cervicis* was partially exposed from the ventro-medial edge of *complexus* by blunt dissection. The insertion of *complexus* onto the occipital crest was cut and *complexus* was reflected laterally to expose both *biventer cervicis* and *splenius*.

Bipolar silver wire electrodes were placed onto one or two of the right or left *complexus*, *splenius*, *biventer cervicis* or *rectus capitis ventralis lateralis* muscles. The collection of data was phase-locked to the sinusoidal VEST by the computer program. Multi-unit electromyograms (EMG) were recorded with high impedance preamplifiers (Grass, model HIP511E), differentially amplified (amplification 10 000 to 50 000) and filtered (pass-band 30 Hz to 10 kHz) (Grass amplifiers model P511F). The incoming polyphasic multi-unit signal of EMG activity thus recorded was digitized with 12 bit precision at 4 kHz by the analogue-to-digital converter of the CED 1401 Programmable Interface (CED, Cambridge, England), and passed to the Tandon PCA30 personal computer which was used to control the experiment and collect the data. The computer then rectified these samples by replacing each with its modulus to produce an array of positive integers. These were then added together in batches of a calculated number to produce an array of 250 grouped samples so that the numbers in the array represented the EMG activity over exactly one vestibular cycle. The number of samples summed in each address of the array depended on the VEST frequency. Thus at 0.4 Hz, the most commonly used VEST frequency, one

vestibular stimulus cycle lasts 2.5s. Ten thousand samples would be digitized by the analogue-to-digital converter during this period of time, so each 'bin' of the array would contain 40 samples of EMG activity, the equivalent of the sum of EMG activity during a period of 10 ms. This treatment produces a convenient measure of the EMG activity recorded from an individual electrode pair.

Cycle histograms (CHSTs) of the rectified EMG activity were built up by averaging over 24 vestibular stimulus cycles (Donaldson and Knox, 1991; Donaldson and Hawthorne, 1976). The raw data from an individual stimulus cycle was not stored. CHSTs were collected in groups of eight. An experiment consisted of one CHST of EMG activity during VEST alone (which acted as a control) and seven CHSTs of the EMG activity produced by VEST plus IEM. The parameters of the eye movement imposed during an individual CHST were controlled by the laboratory computer (see Figures 2.2, 2.3 and 2.4). In some experiments a set of eight CHSTs was made up of four records from one neck muscle and four records from another. In these cases each group of four included a record of the response of the muscle in question to VEST alone. Feedback signals of table position and eye mover position were recorded for the eight stimulus conditions in a similar manner (digitized at a frequency of [250 / period (sec)] Hz and passed to the laboratory computer. At 0.4 Hz, the most commonly used vestibular stimulation frequency, this gives 100 Hz), before collection of EMG data and not simultaneously with it.

Because muscle activity and responses to identical stimuli may vary over time, a technique of interleaved collection of data was used (Henry et al, 1973; Donaldson and Nash, 1975). The EMG activity produced during one vestibular stimulus cycle for each of the eight stimulus conditions (1×VEST alone, 7× VEST+IEM) was collected and stored separately. The whole sequence of eight was repeated 24 times to produce eight CHSTs of averaged EMG activity collected over effectively the same time period, i.e. interleaved in time (Donaldson and Long, 1980). Possible effects produced by the order in which the different stimuli were placed within the group of eight were minimised by altering the presentation order in a pseudorandom manner.

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                    MAIN MENU

Parameters to be set

Display and collect data

File name

Clear histograms

A Collect analogue signals

Examine current data - not available at present!

View data read from disk file

X Plot data

List current directory

Zero all eye-movement orientations

              Q to return to operating system
```

Figure 2.2. Main menu of control program (TUNEMG2) used for electrophysiological experiments. Sub-menus and actions are selected by pressing the initial character of the action required: P, display 'Set parameters menu' (Figure 2.3); D, begin experiment; F, write experimental file to disk; C, clear experimental data and/or analogue records; A, begin collection of analogue information (table and eye-mover position signals); V, view currently stored data; X, download experimental data to HPGL plotter for on-line plot of results; L, list directory to which experimental files are being written; Z, set all eye-movements to same direction (vertically upwards); Q, exit program.

```
                    SET PARAMETERS

Number of sweeps        24

Period of table in sec.         2.5   DAC clock tick rate in msec  10

Number Of interleaved PSTHs 8

Range of orientations, Low (CR) High (CR) 0 360

Histogram settings menu

Set sine Wave for eye-movement

D  Current directory is  C:\DATA

Set eye-mover initial orientation

V Drive the vestibular turntable?   TRUE

Amplitude of table movement (%)   100%

PLotter pen velocity, cm/sec    40
                          Q to leave menu
```

Figure 2.3. Set parameters sub-menu of control program. N, set number of sweeps for a particular experiment (this was usually 24); P, set period of sinusoidal drive signal to vestibular turntable (DAC clock tick rate was adjusted automatically); N, number of interleaved cycle histograms (CHSTs) (4 or 8, this was normally set to 8); R, set range of orientations for eye mover (0° to 360° for directional tuning experiments, 90° to 90° for artificial VOR experiments; H, sub-menu for adjusting parameters of eye movement for an individual CHST (see Figure 2.4); W, sub-menu for adjusting the parameters of the artificial VOR for an individual histogram (see Figure 2.4); D, alter directory to which data files are to be written; S, adjust initial orientation of eye-mover to vertical eye movement (default position) via drive signals sent to the stepper motor; V, turn the vestibular turntable on or off; A, adjust the amplitude of the sinusoidal drive signal sent to the vestibular turntable; Q, return to main menu.

```
                                PSTH 2

Select another PSTH to set

Eye movement on this PSTH? TRUE

Orientation of eye-movement 0.0

Amplitude of eye-movement in percent  100

Rise time of eye-movement in clock tics 20

Hold time of eye-movement in clock tics 100

Delay to start of eye-movement in clock tics 0

P Delay to start of PSTH and analogue in clock tics 0

C Channel for EMG 1 = ADC2, 2 = ADC3  1

Global setting. Set ALL 8 eye-movts. to the values now displayed

Q To return to Set Parameters Menu


                        SINE WAVES FOR EYE-MOVEMENT

Serial number of PSTH for settings ( 1 to 8) 2

Amplitude of sine for eye-movement 100

Phase relative to table 20


Q To return to Set Parameters Menu
```

Figure 2.4. Histogram settings menu (top part of Figure) and settings menu for artificial VOR (bottom part of Figure). Key to histograms settings menu : S, select settings menu for a different histogram; E, turn eye movement on or off; O, select direction of eye movement (0° - 360°); A, amplitude of trapezoidal eye movement (sIEM); R, set velocity of trapezoidal eye movement (sIEM); H, time of hold portion of trapezoid; D, time during vestibular stimulus cycle of imposition of sIEM; P, not used; C, set analogue-to-digital input channel for data collection; G, set all histograms to values set for an individual histogram; Q, exit menu. Key to artificial VOR settings menu: S, select histogram; A, set amplitude of aVOR (0%-100%); P, set phase of aVOR (0°-360°); Q, exit menu.

2.2.5 Analysis of records

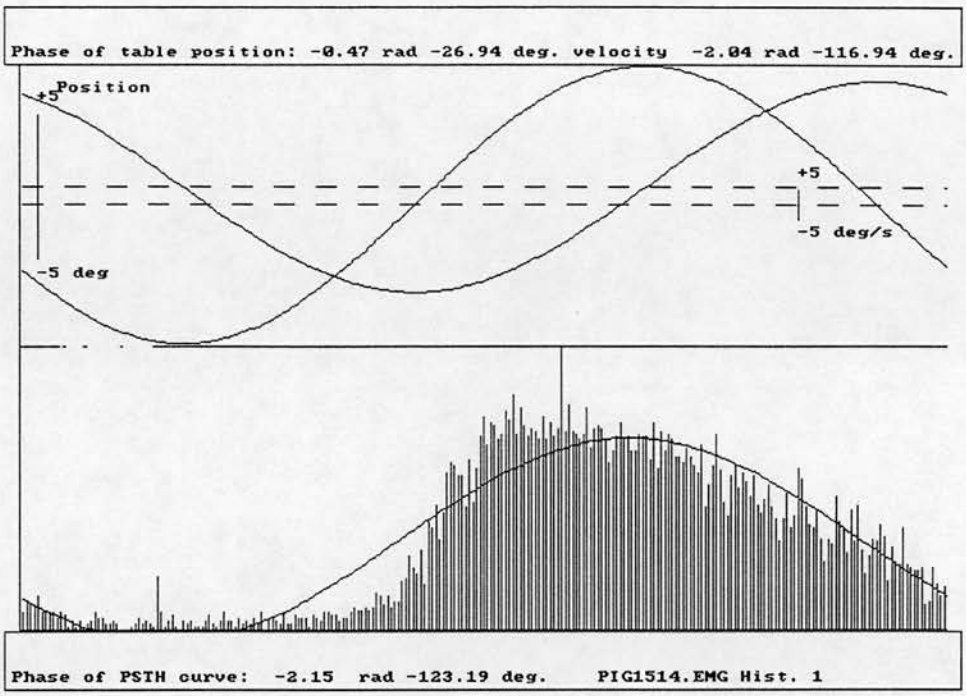
The effect of IEM on the EMG activity of a particular neck muscle during VEST was analysed off-line with a separate program. Sinusoids of the frequency used for VEST were fitted to the analogue records of table position and to the CHSTs of the EMG activity during VEST (with or without IEM). Because the required frequency was known, an analytical method (modified from that of Arzi and Magnin, 1989) was used to solve the differential equations that describe a sinusoid with a minimum sum-of-squares of deviation from the data. This overcame the problems of scaling the CHSTs with 250 bins of data to 256 bins (the nearest power of two) necessary for a fast Fourier transform to fit sinusoids to the data. From the best-fitting sinusoid for table position the corresponding sinusoid for table velocity was obtained by differentiation (see Figure 2.5). Because the head moved with the table, table velocity was equivalent to head velocity. Where there was EMG activity during only a part of the vestibular stimulus cycle, only the bins of the CHST corresponding to the response were used to fit the sinusoid to the CHST (see Melvill Jones and Milsum, 1970, for a discussion). The phase of the neck muscle response was defined as the difference between the phases of the sinusoids fitted to the response and to table velocity or table position. Phase lead was taken as corresponding to occurrence earlier in time. Gain was calculated as the percentage modulation of the mean EMG activity per degree of head movement (used when studying the frequency response) and as (amplitude of sine fitted to response) / (amplitude of sine fitted to table velocity) in units of mV / (deg. s^{-1}). The d.c. level calculated from the sinusoid fitted to the EMG activity was used as an estimate of the mean EMG activity (see Figure 2.5).

The size of the response to IEM (used to calculate the ratio: 'EMG to control') was measured by summing the amount of EMG activity in a time-window (a number of CHST bins) containing the response. The same time-window was used to study the response in all eight CHSTs. In some experiments the modulation of the EMG activity by the stimulus was calculated by subtracting from each bin of the CHST the average background voltage during the part of the cycle when the muscle was not active.

2.2.6 Statistical tests

Tests of the statistical significance of the effects of IEM on the EMG activity were made using a method derived from that of Dörrscheidt (1981) and based on the

binomial theorem (see also Ashton et al, 1984a; Ashton et al 1988 and Donaldson and Knox, 1991). A statistical test is necessary to determine whether the response to combined VEST and IEM is different from the response to VEST alone by an amount greater than the variability of the response to a single stimulus and, so, whether one may attribute the change as due to the interaction of the two stimuli being tested. Dörrscheidt pointed out that it is possible to compare corresponding bins of two peristimulus time histograms (a histogram analogous to our CHST, but measuring the number of spikes of a single neurone during a stimulus cycle) without making any assumption about the statistical properties of the processes which generate the response. If the PSTHs contain the same number of trials (presentations of the stimulus) then the null hypothesis is that corresponding bins (of the same time duration) will be expected to represent samples of the same firing rate if a stimulus condition (e.g. IEM in the horizontal plane vs. IEM in the vertical plane, or IEM vs. no IEM) affects the unit in the same way. Thus impulses will be distributed between the selected, corresponding, bins according to a binomial distribution with $P = Q = (1-P) = 0.5$. The probability of observing a distribution between the test bins at least as extreme as that which occurred can then be found from tables of the binomial distribution or may be calculated from a Normal approximation. The same test can be applied to responses of groups of consecutive bins as long as the same number of bins are compared between PSTHs, since grouping bins is equivalent to constructing a PSTH of greater bin-width. The argument can be extended to comparing CHSTs since the EMG activity is merely the combined firing of many muscle fibres represented by a summed amount of voltage. While this voltage will be a combination of repeated firing of individual muscle fibres and recruitment of other fibres, stimuli that affect the response in the same way will not affect the temporal distribution of this activity. Differences between EMG variability and the effect of a stimulus on EMG activity in corresponding bins of CHSTs can, therefore, also be tested by this method. The procedure, however, only allows responses to be tested in pairs. The test used here is single-sided; that is, it indicates the probability that the larger response is not larger than the smaller one, rather than the probability that the two differ. We decided, therefore, to adopt the critical value of P for rejection of the null hypothesis as 0.025. Values of P greater than this led to the conclusion that the responses were not to be judged different.




```
                PIG1514.EMG  Hist. 1
Data fitted from bin 1 to bin 250

Sinusoid for table position:
DClevel:= 4160.2   A:= 24220.5   Phi:=   -0.5   -26.9 deg

Sinusoid for table position:
DClevel:=  -0.26 deg   A:=   9.95 deg   Phi:=   -0.5   -26.9 deg
Maximum velocity:  25.02 deg/sec
Phase of velocity stimulus:  -2.04 -116.94 deg.

Sinusoid for EMG:
DClevel:= 1476.2   A:= 1665.7   Phi:=   -2.2  -123.2 deg
Max voltage.: 101.71 mV at amplifier output.

Change in velocity stimulus:  25.02 deg/sec

Gain:   4.07 mV per degree/sec

"Spontaneous firing level":  90.15 mV.

Press a key to return to menu
```

Figure 2.5. Fitting a sinusoid to data from electrophysiological experiments, produced in the separate analysis computer program (FILEAN2). Upper figure shows graphics display of one cycle histogram (CHST) from a set of eight with vestibular table position and velocity sinusoids shown above and a sinusoid fitted to the CHST. Text screen below shows the analysis data used to produce the fitted sinusoid and to derive values for the gain of the vestibular response of the various muscles studied. Gain (% modulation / degree) was derived from the amplitude of the fitted sinusoid divided by the D.C. level of this sinusoid, multiplied by one hundred and divided by the amplitude of the table.

2.3 RESULTS

Responses to natural vestibular stimulation

The frequency response of the vestibulocollic reflex (VCR) in *complexus* and *splenius* during horizontal vestibular stimulation was studied over a decade of frequencies from 0.2 Hz to 2.0 Hz and is shown in Figure 2.6. The gain of the VCR can be seen to increase with increasing frequency for both muscles whilst the response is approximately in phase with head velocity. At 0.2 Hertz, the VCR gain is low and phase leads head velocity slightly. At 2.0 Hertz the VCR gain has increased approximately ten-fold and the response lags head velocity by a small amount.

Similar studies were made of the VCR during roll tilt for *complexus*, and *biventer cervicis*. Figure 2.7 shows Bode plots of the frequency responses of *complexus* and *biventer cervicis*. As can be seen the responses were very similar to those seen in the horizontal plane, gain increasing with frequency with a phase close to that of the head velocity. The studies of neck muscle frequency responses in both horizontal and frontal planes were made with both eyes free to move (no lens on the eye). No systematic investigation was made into the effect on the frequency responses of holding one eye still with the suction contact lens.

One unusual finding was the 'cut-off' appearance of the electromyographic activity combined with the presence of a number of distinct peaks or bursts of activity (see Figures 2.9 and 2.10). This cut-off activity occurred when there was little or no spontaneous activity in the muscle being tested.

While *complexus*, *splenius* and *rectus capitis lateralis ventralis* showed vestibularly-evoked activity during vestibular stimulation in the horizontal, frontal and sagittal planes, *biventer cervicis* did not respond to vestibular stimulation in the horizontal plane, although there was considerable spontaneous activity in this muscle. *Biventer cervicis* did, however, show vestibularly-evoked activity with vestibular stimulation in the frontal and sagittal planes.

Responses to imposed eye movement alone

No responses to IEM without concomitant natural vestibular stimulation were observed in any of the four muscles tested (Figure 2.8).

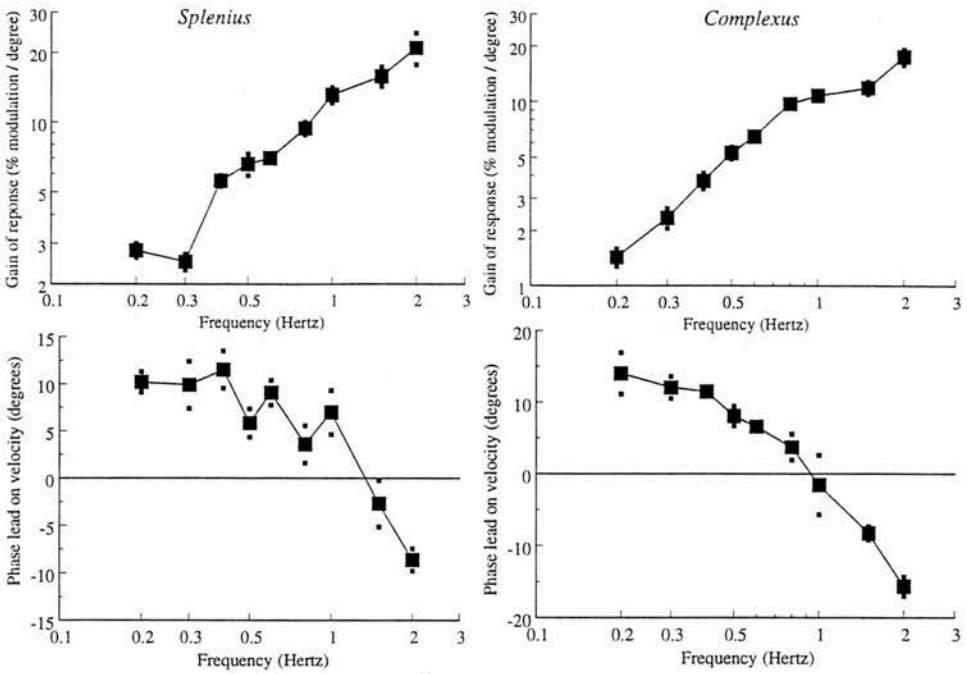


Figure 2.6. Plots of gain and phase of VCR-activity in *splenius* (left-hand side) and *complexus* (right-hand side) during sinusoidal, **horizontal**, vestibular stimulation over a decade of frequency at stimulus magnitudes not exceeding 30°/s (7°/s - 29°/s). Points are mean ± s.e.m. (n=7); scales are log/log for gain, log/linear for phase.

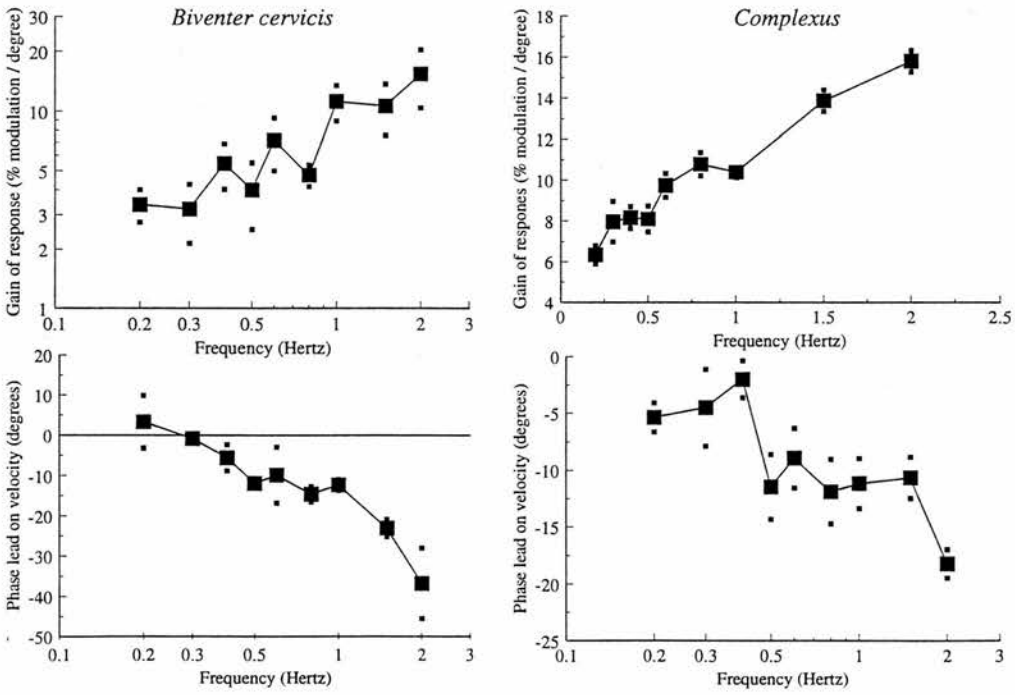


Figure 2.7. Plots of gain and phase in *biventer cervicis* (left-hand side) and *complexus* (right-hand side) during sinusoidal, **frontal** vestibular stimulation over a decade of frequency at stimulus magnitudes not exceeding 30°/s (7°/s - 29 °/s). Points are mean ± s.e.m. (n=8); scales are log/log for gain, log/linear for phase.

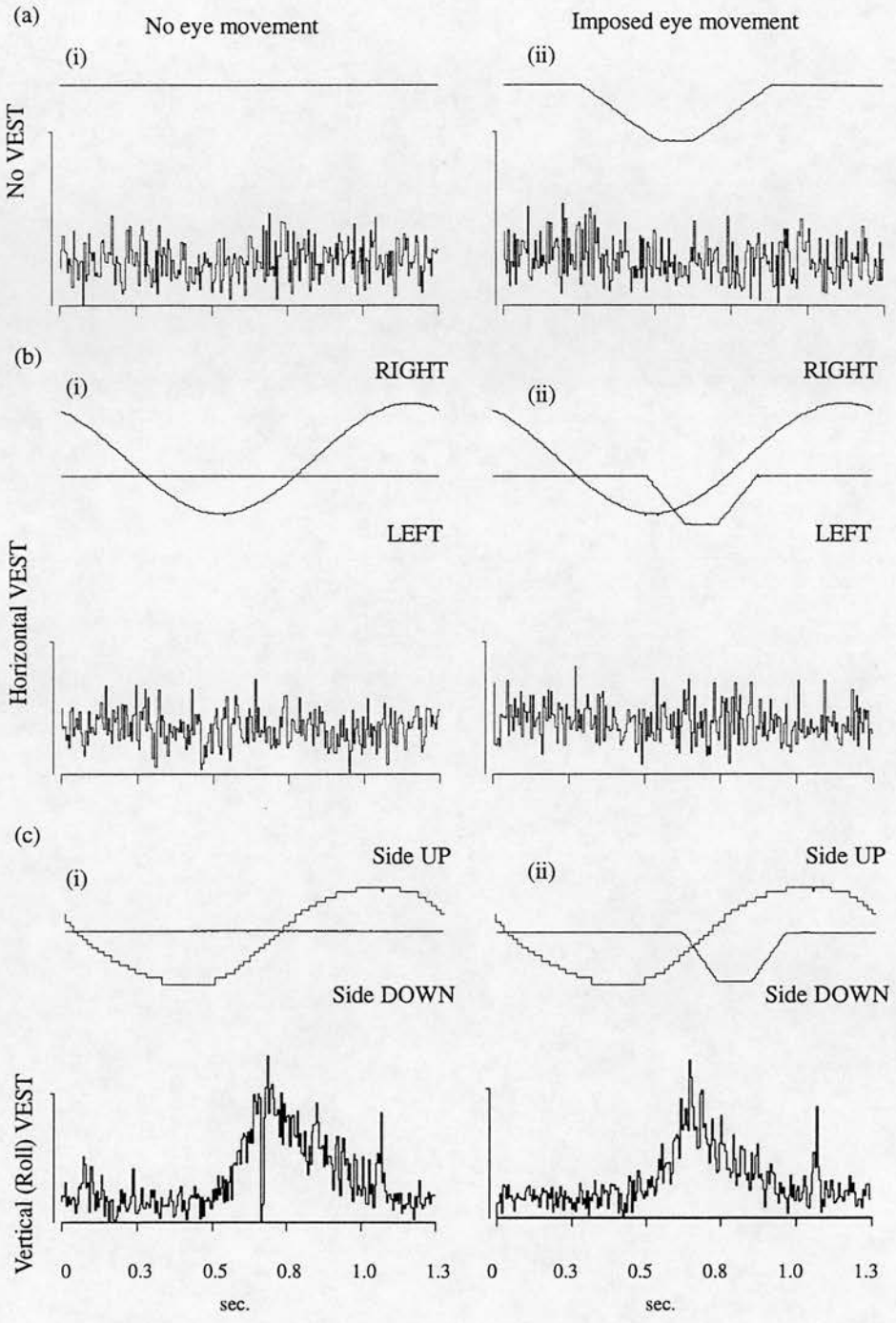


Figure 2.8. Effect of vestibular stimulation (VEST) (no eye movement) (i) and imposed eye movement (IEM) (ii) on the electromyogram (EMG) of the ipsilateral *biventer cervicis*. Six cycle histograms (CHSTs) showing rectified EMG data averaged over 24 sweeps. (a) Spontaneous activity unaffected by IEM. (b) VEST in the horizontal plane produces no effect on the spontaneous activity of the left *biventer cervicis* with or without IEM of the left eye. (c) VEST in the frontal plane (roll tilt) produces a vestibulocollic reflex in the right biventer cervicis which is inhibited by IEM of the right eye. Head position shown by sinusoid (± 8°), eye position shown by trapezoid. Scale bars, 6.4 μV.

Responses to eye movements imposed during horizontal vestibular stimulation

The three muscles that showed a vestibular response to horizontal vestibular stimulation, *complexus*, *splenius* and *rectus capitis ventralis lateralis*, responded to imposed eye movement (IEM) with a modification (usually an inhibition) of the muscle's vestibular response. *Biventer cervicis* showed no effect of IEM on its spontaneous activity with or without horizontal vestibular stimulation (see Figure 2.8).

An example of the response of the left *complexus* to saccadic imposed eye movement (sIEM) of the left eye is shown in Figure 2.9. The response to the sIEM is predominantly an inhibition of the VCR response of the muscle. The effect of the different parts of the imposed trapezoid on the vestibular response of *complexus* was then studied in further detail. Altering the parameters of the imposed trapezoid to increase the time during which the eye was held eccentrically, such that the S1 portion of the trapezoid occurred earlier in time whilst the S3 portion occurred at a point fixed in time, reduced the amount of inhibition produced by IEM (see Figure 2.9). Thus, imposing the S1 movement earlier in the cycle while maintaining the time at which the eye returned to its central position caused a systematic reduction in the amount of inhibition produced by the IEM compared to the control situation in which no eye movement was imposed. Conversely, holding the eye eccentrically throughout the part of the stimulus cycle during which the muscle was active did not produce greater inhibitions in the muscle activity (Figure 2.9). These two tests suggest that the initial, centrifugal eye movement is responsible for producing the observed effects on the vestibular response and not the static position of the eye, S2, or the return eye movement, S3. This experiment was repeated six times on the *complexus* muscle of different preparations, the result was always the same: the S1(initial, centrifugal) portion of sIEM being responsible for the inhibitory effects of sIEM.

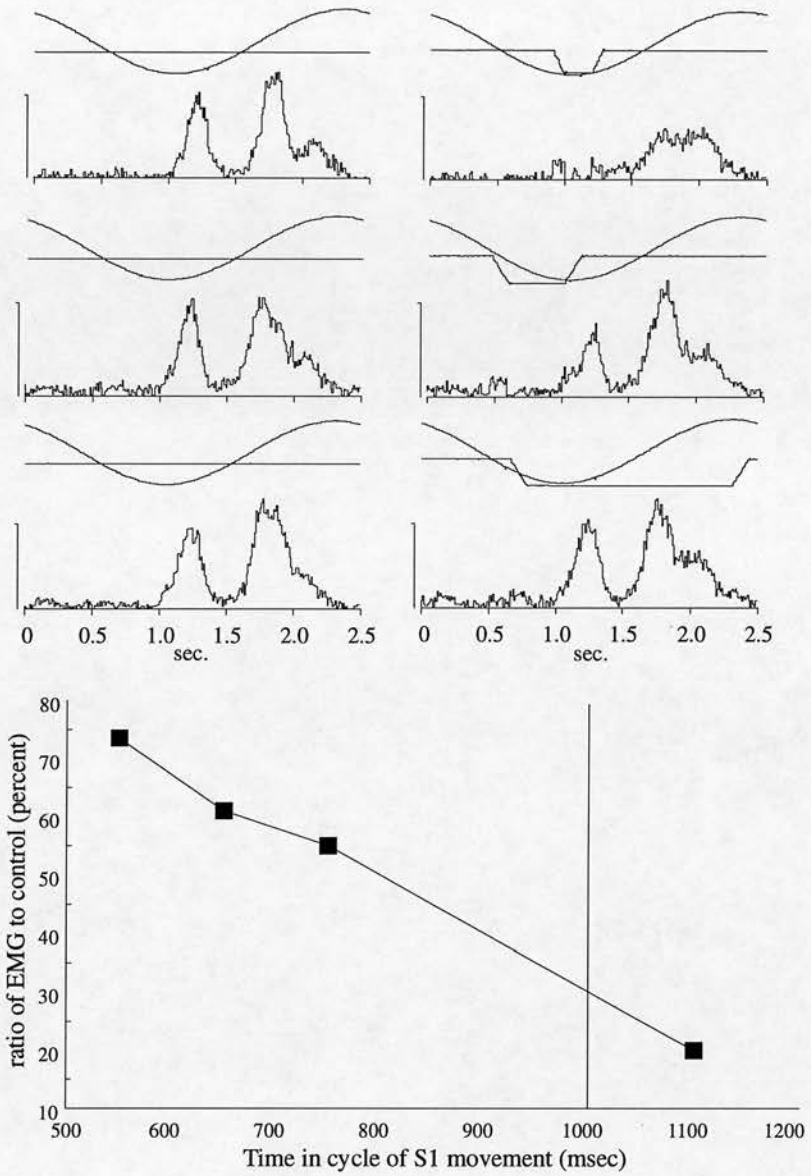


Figure 2.9. Six interleaved cycle histograms (CHSTs) taken from three experiments on the left *complexus* performed to investigate the effects of changing the parameters of saccadic imposed eye movement (sIEM) on the VCR-response. The three left-hand CHSTs show the responses to vestibular stimulation (VEST) alone (the left eye being held still) during the three experiments. The top right-hand CHST shows the response to VEST and the normal sIEM test stimulus (delay = 1.1 sec, hold-time = 200 msec; amplitude = 12.5°) directed towards the beak (right, 270°). The VCR-response is strongly inhibited. The middle right-hand CHST shows a much smaller inhibition when the initial, centrifugal portion of the sIEM (S1) occurs earlier in time (delay = 550 msec). The bottom right-hand CHST shows again a much smaller inhibition of the VCR-response when the eye is held eccentrically over a large portion of the vestibular stimulation cycle. The graph in the bottom half of the figure shows the effect of changes in the VCR-response produced by altering the time at which the initial portion of sIEM (S1) was imposed. As sIEM is imposed closer to the point in time at which the VCR-response of the muscle occurs (shown by the vertical line), the VCR response is increasingly inhibited. CHSTs show rectified EMG data averaged over 24 sweeps, scale bars = 6.5μV.

The effect of different directions, amplitudes and velocities of sIEM was then studied for the different muscles.

The length of time over which the inhibitory response produced by sIEM lasted differed for *splenius* compared to *complexus* and *rectus capitis ventralis lateralis*. *Splenius* showed a marked phasic inhibition shortly after (50 msec) the initial S1 portion of sIEM and, in some cases, a longer lasting inhibition for the remainder of the vestibular activity (see Figure 2.14). *Complexus* and *rectus capitis ventralis lateralis* did not show a marked phasic inhibition during the S1 portion of sIEM, instead both muscles responded to sIEM with a long lasting inhibition, occurring with a similar latency (50 msec) to that seen for *splenius* (see Figures 2.10).

Imposing trapezoidal eye movements in a number of different orbital radii allowed the directional sensitivity of the vestibular activity to be studied. The different muscles studied showed differences in their sensitivity, or 'tuning' to the direction of sIEM. There was also a marked difference in the response of an ipsilateral neck muscle (one on the same side as the eye on which movements were imposed) to sIEM compared to that of a contralateral neck muscle.

The ipsilateral *complexus* was inhibited to the greatest extent by sIEM initially directed towards the beak (labelled as 270° in our co-ordinate system) in the majority of experiments (7/13) (Figure 2.10); however, in two experiments sIEM initially directed vertically downwards (180°) produced the largest inhibition of the muscle's vestibular response (Figure 2.11). sIEM tailwards (90°) inhibited the vestibular response of the contralateral *complexus* by the largest amount in most of the experiments (4/7) (Figure 2.12), but sIEM initially directed vertically upwards (0°) produced the greatest inhibition in one experiment (Figure 2.11). In further experiments the 'tuning' of the directional responses to sIEM was less specific, but still showed the same directional bias. Thus the ipsilateral *complexus* was most inhibited by beakwards and downwards sIEM (180°-270°) in 12 out of 13 experiments and the contralateral *complexus* by tailwards and upwards sIEM (0°-90°) in 6 out of 7 experiments.

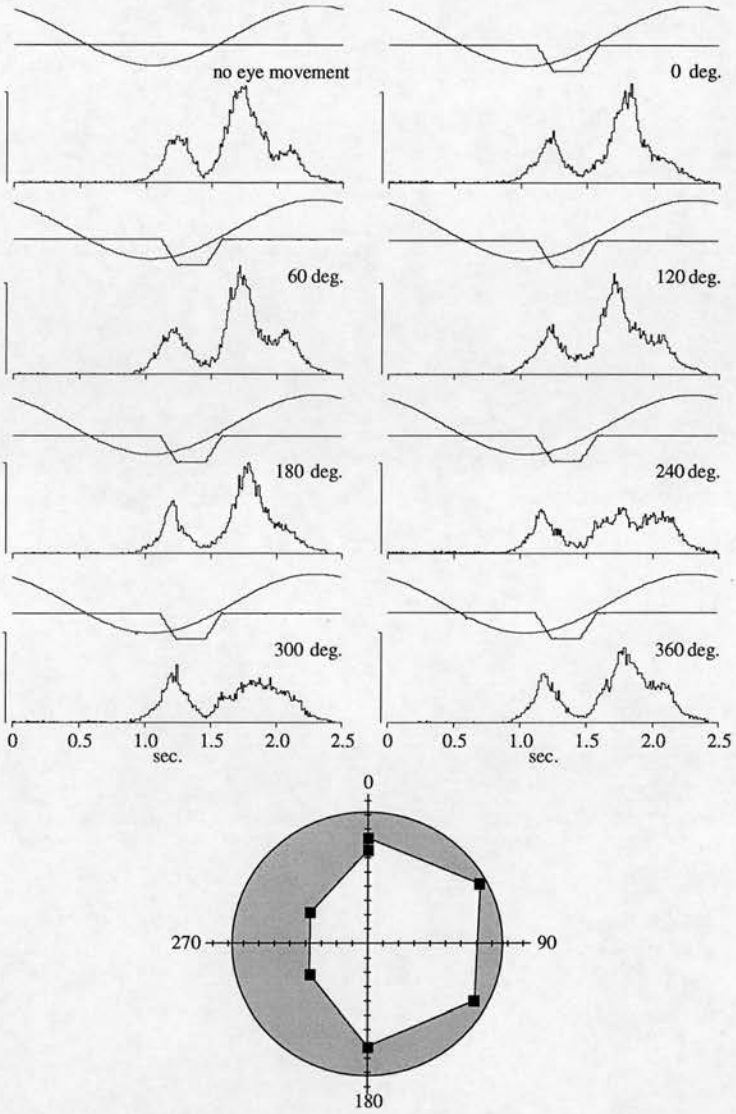


Figure 2.10. Set of eight interleaved cycle histograms (CHSTs) showing the effect of different directions of saccadic imposed eye movement (sIEM) on the electromyographic (EMG) activity of the left *complexus* during horizontal vestibular stimulation (VEST, ±8° at 0.4 Hz). Each CHST represents exactly one cycle of vestibular oscillation. Upward deflection of vestibular table position trace (sinusoid) represents movement to the right (contralateral to the muscle being recorded from). Eye position trace (solid line) shows the time course of sIEM. The top left CHST shows the response to VEST alone (eye held still). The remaining seven CHSTs show the response to VEST and added sIEM directed in various directions: 0°/360° corresponds to sIEM initially directed vertically upwards; 60°, upwards and towards the tail (left); 120°, downwards and towards the tail; 180°, vertically downwards; 240°, downwards and towards the beak (right) and 300°, upwards and towards the beak. Scale bars for CHSTs, 6.4μV. The plot in the lower half of the figure is derived from the eight CHSTs, being constructed from the modulation of the rectified EMG integrated over the same time window, bins 159 to 195 (370 ms), in each CHST. The response to a particular direction of sIEM is plotted as a vector in which the distance of a point from the centre of the plot represents the magnitude of the response, and the angle of the vector shows the direction of sIEM. The circle represents the response to VEST alone (control). The shaded area shows the inhibition produced by sIEM relative to the control response. Scale divisions for polar plot 0.5 μV. sIEM directed towards the beak (240° & 300°) produced the largest inhibitions in the VCR response which were statistically significant ($P<0.005$) differences from all other directions of sIEM.

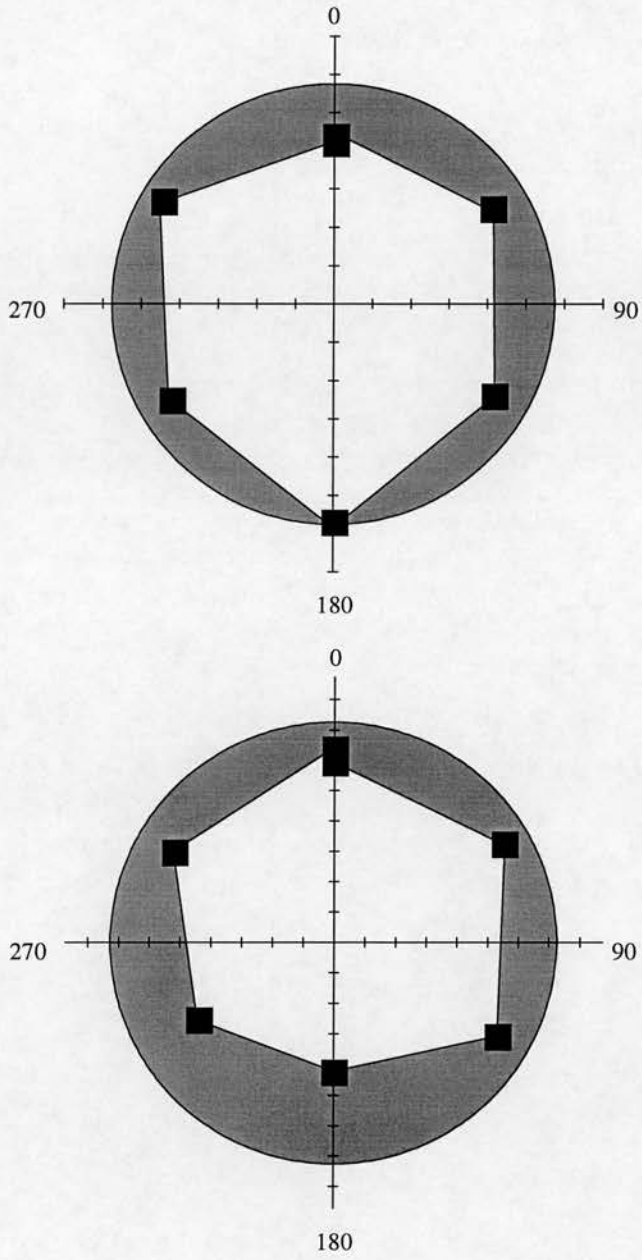


Figure 2.11. Plots of 'directional-tuning' of the effects of saccadic imposed eye movement (sIEM) of the left eye on the VCR response of the left *complexus* (bottom) and right *complexus* (top). Plots are derived in exactly the same manner as described in Figure 2.10. Left *complexus*, response window bins 144-207 (256 ms), scale divisions 0.6μV. sIEM initially directed vertically downwards (180°) produced the greatest inhibition in the VCR response, to 46% of the control value. All directions of sIEM produced statistically significant ($P<0.005$) reductions from the control value (eye held still), and sIEM at 180° produced a reduction that was statistically significant ($P<0.005$) from all other directions of sIEM. Right *complexus*, response window bins 154-181 (112 ms), scale divisions 0.7 μ V. sIEM directed vertically upwards (0°) produced the largest inhibition in the VCR response, to 75% of the control value. All directions of sIEM produced statistically significant ($P<0.025$) reductions from the control value (eye held still), and sIEM at 0° produced a reduction that was statistically significant ($P<0.005$) from all other directions of sIEM.

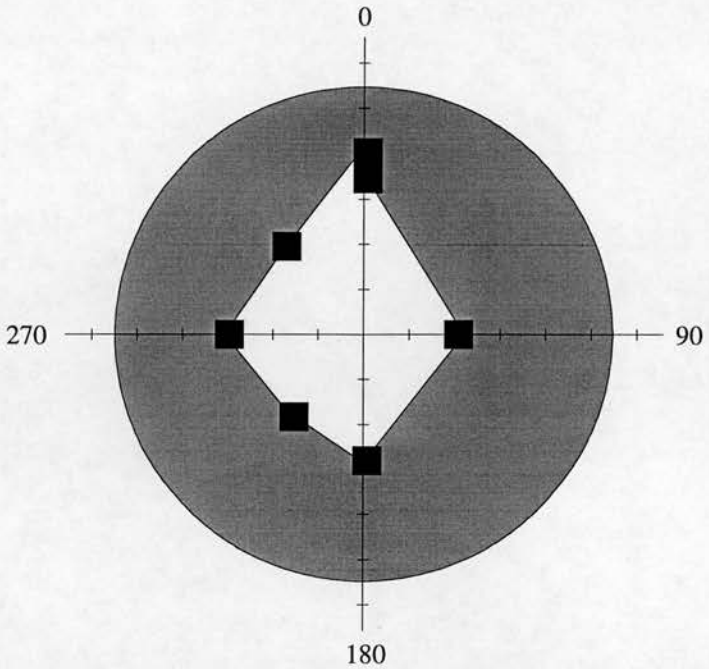


Figure 2.12. Directional-tuning plot of the effect of saccadic imposed eye movement (sIEM) on the right *complexus* during horizontal, vestibular stimulation. The plot is constructed as described in Figure 2.10. Response window bins 131 to 204 (740ms), scale divisions 0.1μV. sIEM initially directed towards the tail (left, 90°) produced the largest reduction in the VCR response, to 27% of the control value (eye held still). All directions of sIEM produced statistically significant ($P<0.025$) reductions from the control value (eye held still), and sIEM at 90° produced a reduction that was statistically significant ($P<0.025$) from all other directions of sIEM.

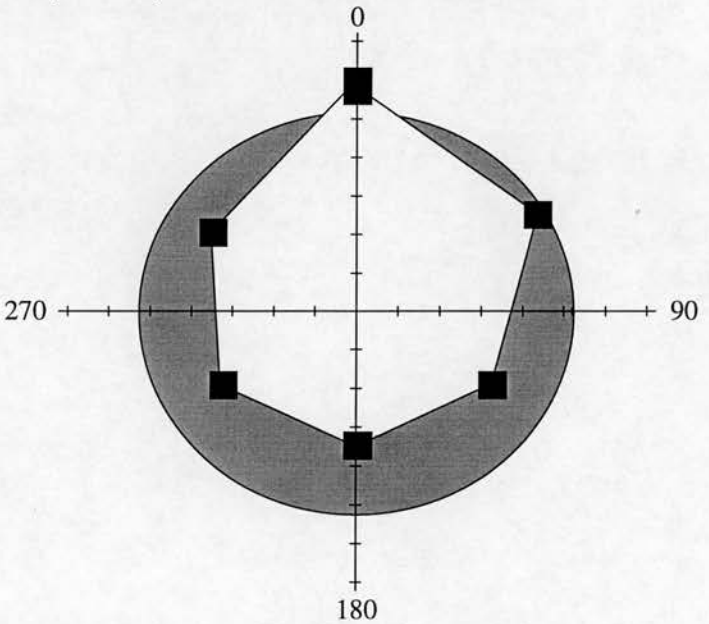


Figure 2.13. Plot of directional tuning of the effects of saccadic imposed movements of the left eye (sIEM) on the VCR response of the left *rectus capitis ventralis lateralis*. The plot is derived in exactly the same manner as that of Figure 2.10. Response window bins 122-150 (290 ms), scale divisions, 1.7μV. The VCR response to sIEM directed vertically downwards (180°) or downwards and towards the left (beak, 120°) or right (tail, 240°) are significantly different from the control (eye held still) response and the VCR response to all other directions of sIEM ($P<0.025$). Similarly the VCR response to sIEM directed vertically upwards (0°/360°) is significantly larger than the control VCR response ($P<0.025$).

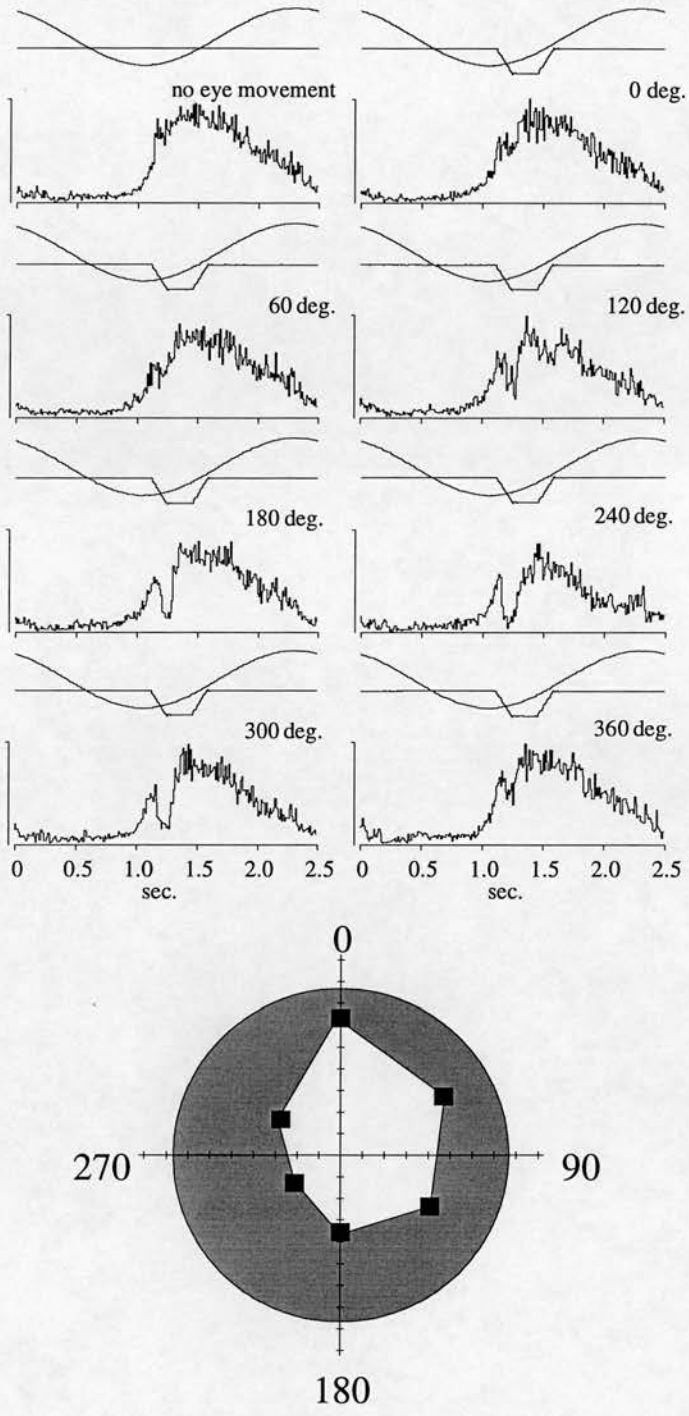


Figure 2.14. Set of eight interleaved cycle histograms (CHSTs) showing the effect of different directions of saccadic imposed eye movement (sIEM) on the electromyographic (EMG) activity of the left *splenius* during horizontal, sinusoidal vestibular stimulation (±8° at 0.4 Hz). Directions of sIEM and construction of polar plot (lower half of figure) are as those described in Figure 2.10. Time window from which modulation of rectified EMG activity used to construct the polar plot was calculated was 115 to 135 (210ms), scale divisions 0.5 μV. Scale bars for CHSTs 6.25 μV. sIEM directed towards the beak (240° & 300°) produced the largest inhibitions in the VCR response, these were statistically significant ($P<0.005$) differences from all other directions of sIEM. sIEM produced statistically significant reductions ($P<0.005$) in the VCR response compared to the control (eye held still) VCR response.

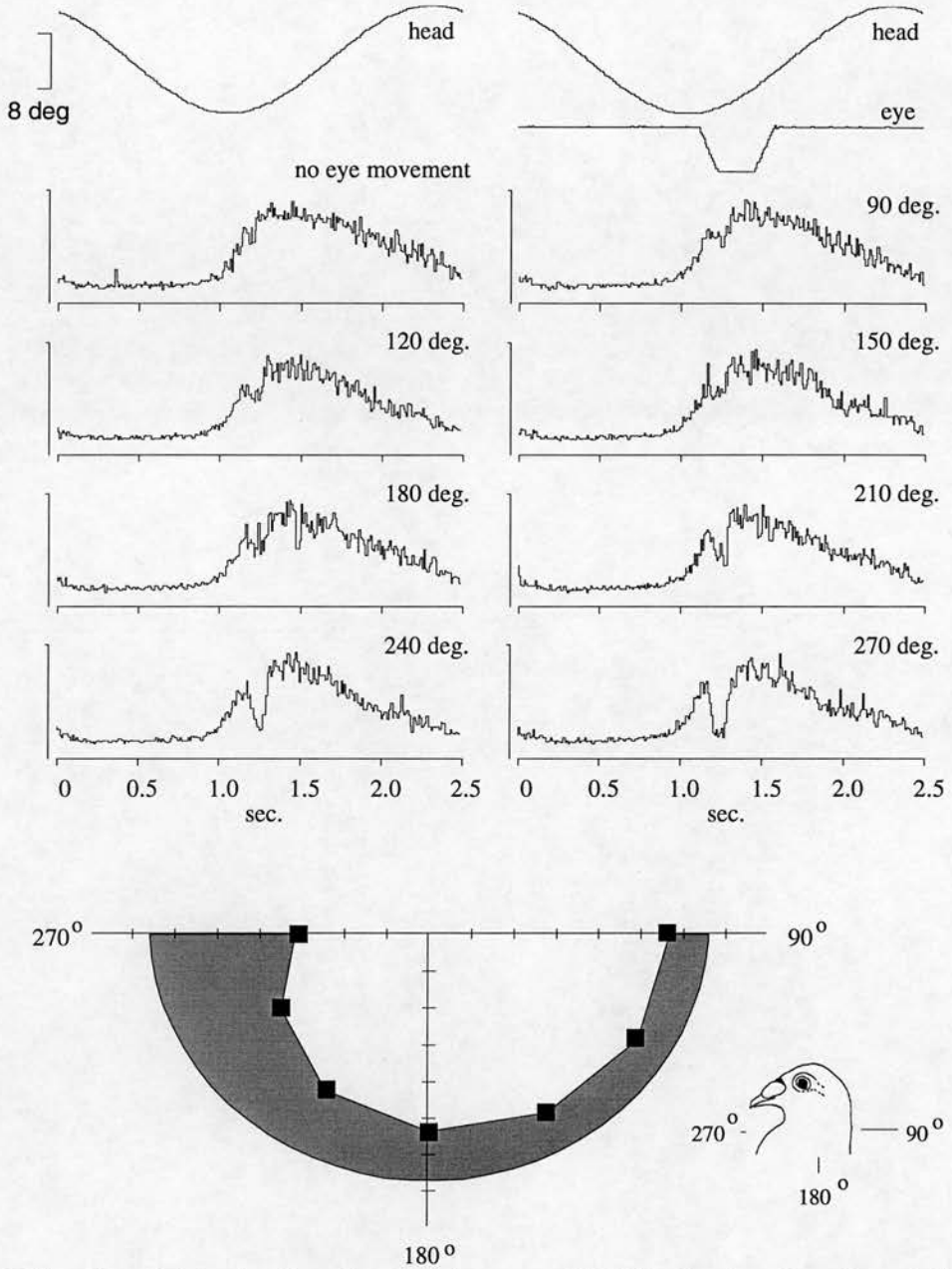


Figure 2.15. Set of eight interleaved cycle histograms (CHSTs) showing the effect of different directions of saccadic imposed eye movement (sIEM) on the electromyographic (EMG) activity of the left *splenius* during horizontal, sinusoidal vestibular stimulation (±8° at 0.4 Hz). This set of histograms and the polar plot of directional tuning constructed from them is an example of an experiment to investigate the 'fine tuning' of the response of the muscle, with sIEM directed in orbital radii from towards the left (tail, 90°) to towards the right (beak, 270°). The experiment was performed on the same muscle as the experiment shown in Figure 2.14. Scale bars for CHSTs 6.4 μV. Response window bins for polar plot 115 to 135 (210 ms), scale divisions 0.6 μV. sIEM directed towards the right (beak, 270°) produced the largest inhibition in the VCR response which was a statistically significant ($P<0.005$) difference from all other directions of sIEM. All directions of sIEM produced statistically significant ($P<0.005$) reductions of the VCR response from the control (eye held still).

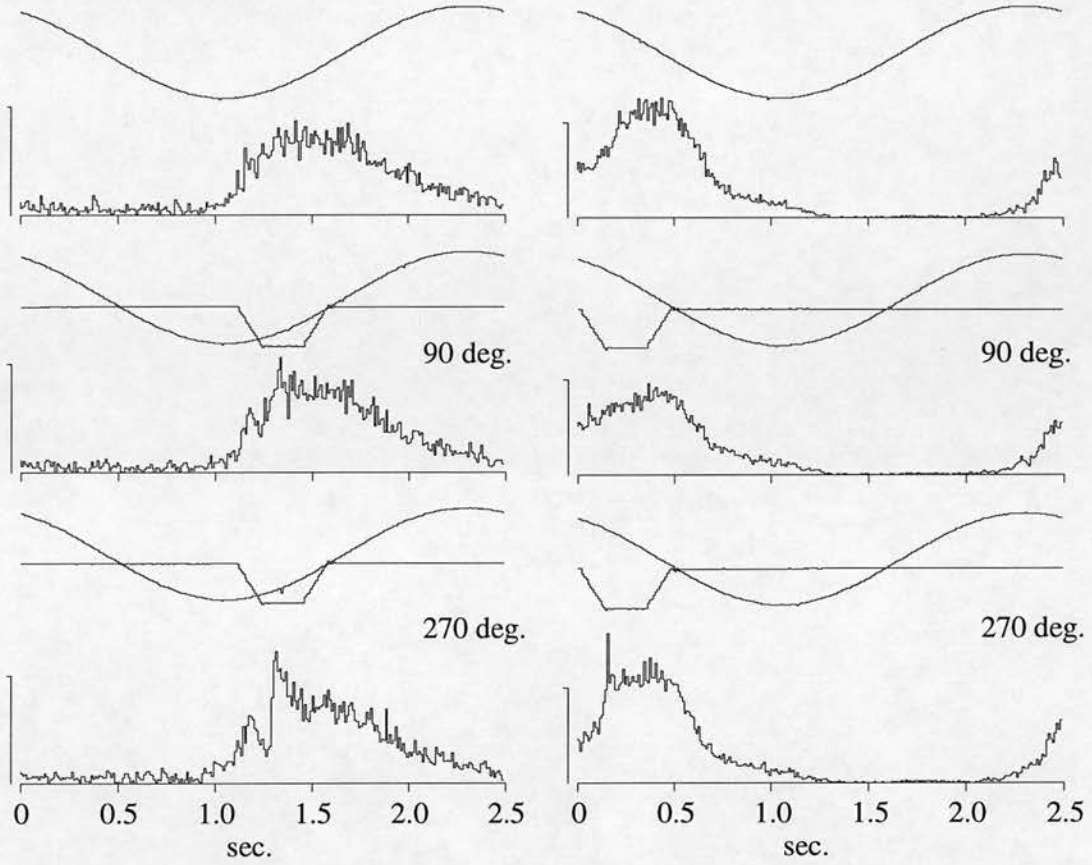


Figure 2.16. Six interleaved cycle histograms taken from a set of eight showing the effect of different directions of saccadic imposed eye movement (sIEM) of the left eye on the electromyographic activity of the left *splenius* (left-hand side) and the right *splenius* (right-hand side) during horizontal, sinusoidal vestibular stimulation (±8° at 0.4 Hz). The top two CHSTs show the response to vestibular stimulation (VEST) alone. The middle two CHSTs show the effect of VEST with added sIEM directed towards the tail (left, 90°). sIEM can be seen to inhibit the VCR-response of the right *splenius*, but to have little effect on the left *splenius*. The bottom two CHSTs show the effect of VEST with sIEM directed towards the beak (right, 270°). The left *splenius* is initially inhibited and then shows a longer latency, or rebound, excitation whereas the right *splenius* shows only slight excitation with a similar latency to the excitation seen in the left *splenius*. Scale bars for CHSTs, 6.4 μV.

The ipsilateral *rectus capitis ventralis lateralis* was inhibited to the greatest extent by sIEM initially directed downwards (180°) with sIEM directed vertically upwards producing the smallest degree of inhibition (Figure 2.13). This directional-tuning was seen in 4 out of 6 experiments on the ipsilateral *rectus capitis ventralis lateralis*.

The ipsilateral and contralateral *splenius* showed 'directional tuning', in the horizontal plane, that was similar to that of *complexus*. The vestibular response of the ipsilateral splenius was inhibited to the greatest extent by sIEM beakwards (270°) in 12 of 17 experiments (Figures 2.14, 2.15 & 2.16) whereas that of the contralateral splenius was most inhibited by sIEM tailwards (90°) in 4 out of 5 experiments (Figures 2.16 & 2.17). The effect of sIEM in different directions on the vestibular

response of *splenius* was notably sharper and more sensitive than that of *complexus*; however, the ipsilateral splenius also showed reductions in vestibular activity with sIEM directed vertically downwards (180°) and the contralateral *splenius* showed reductions with sIEM directed vertically upwards (0°/360°). While these reductions were not the largest seen in a particular 'tuning' experiment, they are similar to the effects of vertically directed sIEM seen in the ipsi- and contralateral *complexus*.

All muscles showed systematic increases in the magnitude of the inhibition of their vestibular responses with increasing amplitudes or velocities of sIEM. These effects were also affected by the direction of the sIEM, suggesting that the inhibitory effects of amplitude/velocity were added to those of direction (see Figures 2.18 and 2.19).

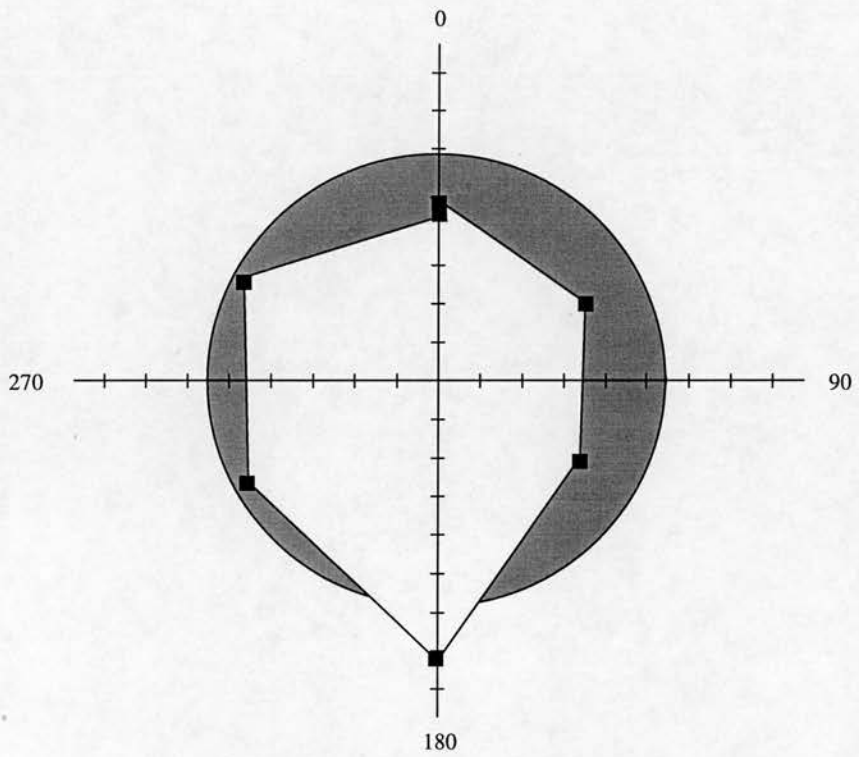


Figure 2.17. Polar plot to illustrate the effect of different directions of saccadic imposed eye movements (sIEM) of the left eye on the VCR-response of the right *splenius* during horizontal, sinusoidal vestibular stimulation. Plot is derived as described in Figure 2.10, response window bins 123 to 134 (120 ms), scale divisions 0.12 μV. sIEM upwards and towards the tail (0°, 60° & 120°) produced the largest inhibitions in the VCR response which were significantly different ($P < 0.025$) from sIEM directed in all other directions and the control (eye held still) VCR response. sIEM directed downwards (180°) produced an increase in the VCR response above that seen in the control (eye held still), which was significantly greater than the control response ($P \ll 0.005$).

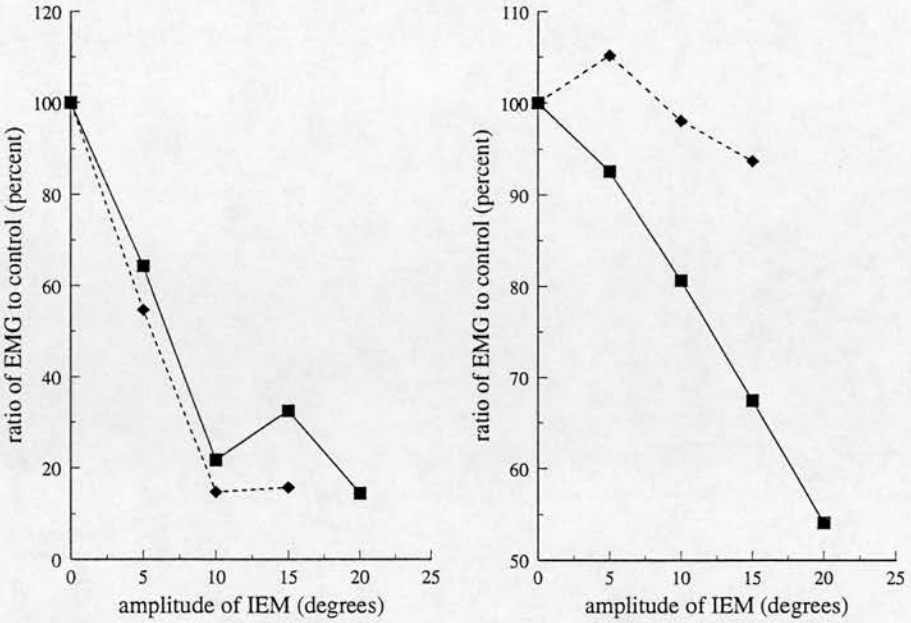


Figure 2.18. Effect of change of amplitude (5° to 20°) of horizontal saccadic imposed eye movement (sIEM) of the left eye towards the left (tail, squares) or right (beak, diamonds), velocity held constant at 115°s^{-1}, on the left (left-hand graph) and right (right-hand graph) *complexus*. Graphs show the ratio of the modulation of the averaged electromyographic (EMG) activity during combined horizontal, sinusoidal, vestibular stimulation (VEST) and sIEM, to the modulation of the EMG activity during VEST alone (control), plotted against the amplitude of sIEM. The magnitude of the reduction in EMG activity is dependent on the direction of the eye movement, as well as on its amplitude with movements towards the right (beak) having the largest effect on the left *complexus* (ipsilateral muscle) and movements towards the left (tail) having the largest effect on the right *complexus* (contralateral muscle). The inhibition of the VCR response produced by sIEM directed towards the right and left was statistically different ($P<0.005$) for all amplitudes of sIEM for both muscles.

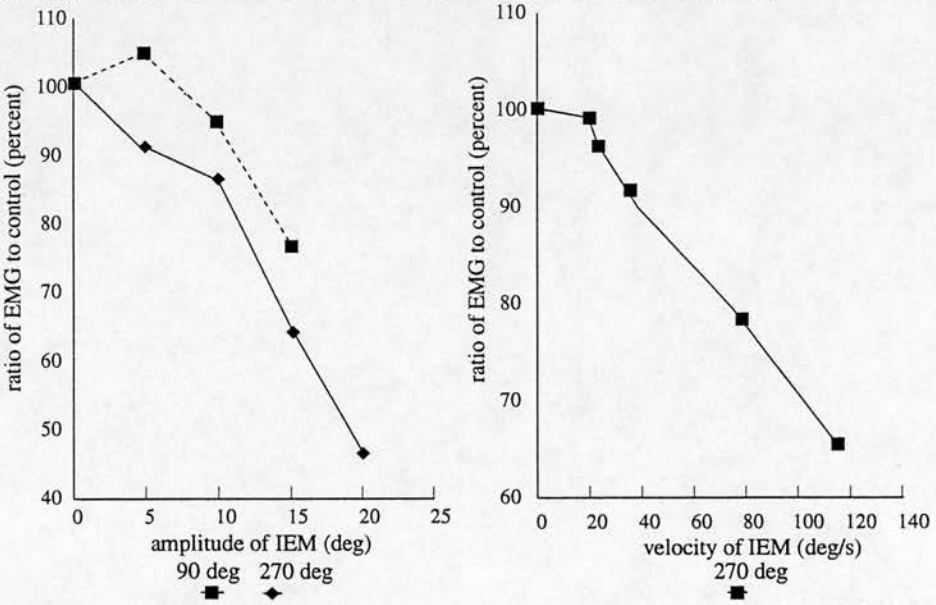


Figure 2.19. Effect of change of amplitude (left-hand graph, 5° - 20°) and velocity (right-hand graph, 28°s^{-1} -115°s^{-1}) of sIEM of the left eye on the left *splenius*. Graphs derived as described in Figure 2.18. sIEM directed towards the right (beak, 270°) inhibits the VCR response of the left *splenius* more than sIEM directed towards the left (tail, 90°). These differences are statistically significant ($P<0.025$) for each of the amplitudes used.

Responses to the artificial VOR

The second type of IEM used was that described as the artificial VOR (aVOR) in which a movement was imposed on one eye in a manner that mimicked the slow phase of the vestibuloocular reflex (VOR). The peak table velocity during the imposed sinusoidal vestibular stimulation was normally 22°/s thus this was also the peak head velocity. The compensatory aVOR was defined as an imposed sinusoidal eye movement with a peak speed of 22°/s, but in the opposite direction to the head. Thus the compensatory aVOR imposed a movement equivalent to a slow phase VOR with a gain of -1.0. The most immediately noticeable difference between the effects of sIEM and the aVOR was that the aVOR produced inhibition over the whole of a muscle's vestibular response rather than only a part of it (Figure 2.20).

Effect of combined velocity and amplitude errors of the aVOR

Altering the peak speed of the imposed eye movement from 6°/s to 41°/s allowed combined velocity and amplitude errors to be imposed during the aVOR. The response to horizontal vestibular stimulation alone was treated as an aVOR with a peak speed of 0°/s. Imposing velocity/amplitude errors with the aVOR produced systematic changes in the vestibular response of all the muscles that responded to horizontal vestibular stimulation (*splenius*, *complexus* and *rectus capitis ventralis lateralis*). Comparing the effects with those seen when imposing an aVOR at the compensatory velocity, an aVOR slower than compensatory increased the vestibular response of a muscle while an aVOR with a faster speed than required for compensation reduced or inhibited a muscle's vestibular response (Figure 2.20).

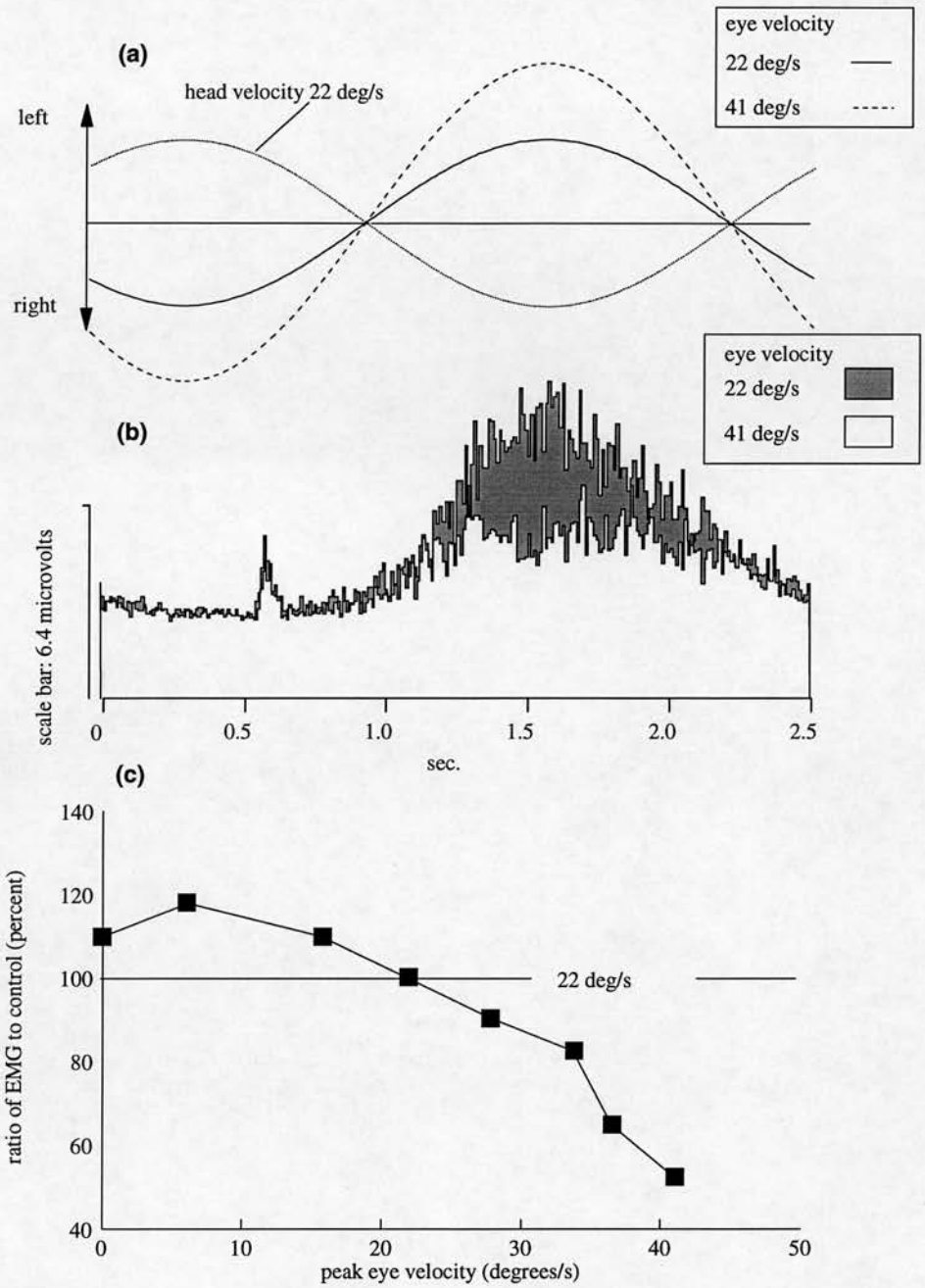


Figure 2.20. Experiment using 'artificial VOR' with velocity errors as explained in text. (a) Sinusoids showing head velocity (dotted line) during a vestibular stimulus cycle (± 8° at 0.4 Hz) and imposed eye velocity at the compensatory velocity (solid line) and at a velocity approximately twice compensatory (dashed line). (b) Superposed records of EMG modulation of the left *splenius* during the 'artificial VOR' from a set of interleaved histograms averaged over 24 repetitions. The shaded area represents the reduction in the VCR response in *splenius* when the imposed eye velocity is almost twice that required to produce a compensatory VOR. Scale bar 6.4 μV. (c) Results from the same experiment as (b). Plot of the ratio of the VCR response of the left *splenius* during imposed movements of the left eye at various velocities, to its VCR response during the compensatory aVOR (eye velocity, $22°s^{-1}$). The VCR response is reduced as eye velocity increases above the $22°s^{-1}$ required for compensation of the vestibular stimulus, and increases as eye velocity decreases below that required for compensation.

The results of each experiment in which changes in the aVOR velocity were imposed on one eye were collated. Linear regressions were constructed of: (ratio of responses at various velocities of aVOR to control response, the compensatory aVOR) to (velocity of aVOR); the correlation coefficients were greater than r=0.7 (r^2>0.5) in a large number of experiments (30/40). Analysis of covariance of the regression data for pairs of experiments from a particular muscle with the widest spread of parameters and/or largest difference in regression slopes suggested that the regression lines form a group homogeneous in slope, with a mean slope of approximately -1 for each of the three muscles tested. The values of the correlation coefficients for the pooled data from each of the three muscles gives probabilities of much less than 0.1% that inhibition of neck muscle vestibular activity is independent of the imposed eye velocity. Analysis of covariance for the pooled regression data from each of the three muscles showed no significant difference between the slopes of the combined regression data for the individual muscles. Combining the data from a total of 23 experiments on *complexus*, *rectus capitis ventralis lateralis* and *splenius* produced the regression plotted in figure 2.21 which has a slope of -1.13. The value of the correlation coefficient (r) of 0.78 for 176 degrees of freedom gives P<0.001. This leaves no doubt about the statistical correlation between EMG activity in a muscle and velocity of imposed eye movement. The combined regression slope of -1.13 means that for each degree-per-second increase in velocity of eye movement during the aVOR, there was a reduction of about 1% in the vestibularly-evoked EMG activity in *splenius*, *complexus* or *rectus capitis ventralis lateralis*.

The effect of the aVOR with velocity/amplitude errors on the contralateral muscle as opposed to the ipsilateral muscle was similar, but the slope of the regression line was not as large as that seen in the ipsilateral muscles. The phase of a muscle's vestibular response remained virtually unchanged during the aVOR at any velocity (Figure 2.22).

Effect of errors in the phase of the aVOR

Altering the phase of the aVOR whilst maintaining the amplitude, and therefore the velocity, at that of the compensatory aVOR also produced changes in the vestibular response of those muscles responding to horizontal vestibular stimulation. For descriptive purposes, phase lead indicates occurrence earlier in time, and is denoted as positive. The results of imposed phase errors were not as consistent as those seen with imposed velocity and amplitude errors. However a general trend emerged for *splenius* and *complexus*. As phase lag increased, the

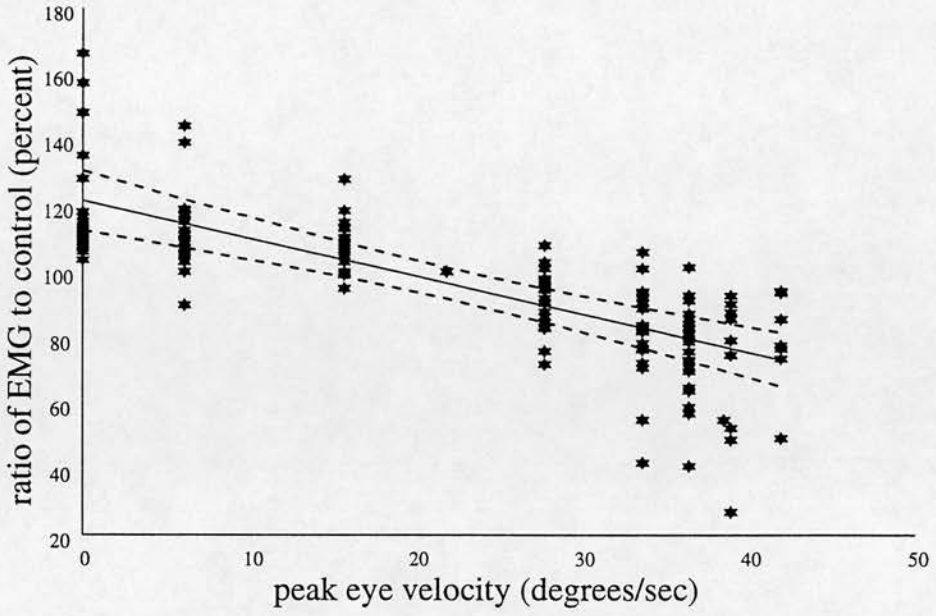


Figure 2.21. Data from 23 experiments in which an 'artificial VOR' (aVOR) was imposed on the left eye at different amplitudes/velocities as described in Figure 20 on the left *complexus*, *splenius* or *rectus capitis ventralis lateralis*. Spread of data is shown (filled stars) as well as the linear regression of the pooled data (176 degrees of freedom) (solid line). Dotted hyperbolas are 95% confidence limits for the line. The correlation coefficient is highly significant ($r = 0.78$; $P < 0.001$). The VCR response falls, on average, by about 1% for each $1°s^{-1}$ increase in peak eye velocity during the aVOR.

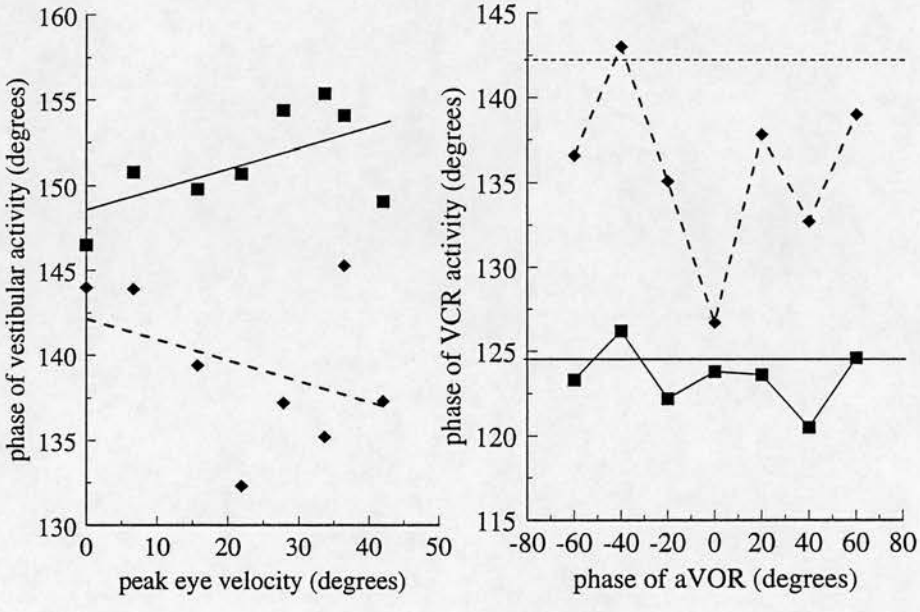


Figure 2.22. Effects on the phase of the VCR response of the artificial VOR (aVOR) with amplitude/velocity errors (left-hand side) or with phase errors (right-hand side) of the left *complexus* (squares, solid lines) and *splenius* (diamonds, dotted lines). Linear regressions (left-hand side graph) constructed from the data showed that there was no statistically significant change in the phase of the VCR response ($r<0.5$, $P>0.05$) during the aVOR with amplitude/velocity errors. The aVOR with phase errors (right-hand side graph) produced small and inconsistent changes in the phase of the VCR response of both muscles. The horizontal lines show the phase of the VCR response during vestibular stimulation alone (no IEM).

vestibular response of a muscle decreased compared to its response when there was no phase error in the imposed sinusoid and as phase lead increased the vestibular response increased slightly above that seen at the compensatory aVOR. In Figure 2.23 the vestibular activity in the left *splenius* (ipsilateral) decreased compared to that at the compensatory aVOR as phase lag increased from 30° to 90° to a minimum of 62% of the activity at the compensatory aVOR. As the imposed aVOR began to lead the compensatory aVOR the vestibular activity increased to a maximum of 110% of the activity at the compensatory aVOR at a phase lead of 60°. The vestibular activity then decreased to slightly below that of the muscle during the compensatory aVOR when the imposed aVOR phase lead the compensatory aVOR by 90°; similar results were obtained in 8 out of 12 experiments on the ipsilateral *splenius*. The vestibular activity when there was no IEM was larger than that at the compensatory aVOR as seen with the amplitude/velocity experiments. A similar, large reduction of vestibular activity in the left *complexus* (ipsilateral) with phase lead can be seen in Figure 2.24. The vestibular activity of *complexus* is reduced to 39% of the activity of the muscle during the compensatory aVOR at a phase lag of 40° and increased to a maximum of 113% of this activity at a phase lead of 20°; similar effects were seen in 4 out of 5 experiments on the ipsilateral *complexus*.

The effect of the aVOR with phase errors on the contralateral *splenius* and *complexus* muscles was very similar to that seen in the ipsilateral muscles, but the reductions in the vestibular activity with phase lag were smaller than those seen in the ipsilateral muscles (Figure 2.25). The effect of the aVOR with phase errors on *rectus capitis ventralis lateralis* was quite different to that seen in *splenius* and *complexus*. The major differences are shown in Figure 2.26 where it can be seen that while phase lags did produce reductions in the ipsilateral muscle's vestibular activity (to a minimum of 24% of the activity during the compensatory aVOR), phase leads also reduced the vestibular activity of the muscle to below that at the compensatory VOR, although this was to a lesser extent (to a minimum of 87%). Conversely, the VCR response of the contralateral *rectus capitis ventralis lateralis* showed slight increases compared to the vestibular activity at the compensatory aVOR with phase lags and large increases with phase leads (to a maximum of 146% of the activity during the compensatory VOR). Furthermore, the VCR response of the ipsilateral *rectus capitis ventralis lateralis* when the eye was held still (VEST alone) was larger than the VCR response during all imposed movements of the left eye, whereas it was always smaller than the VCR response during imposed eye movements in the

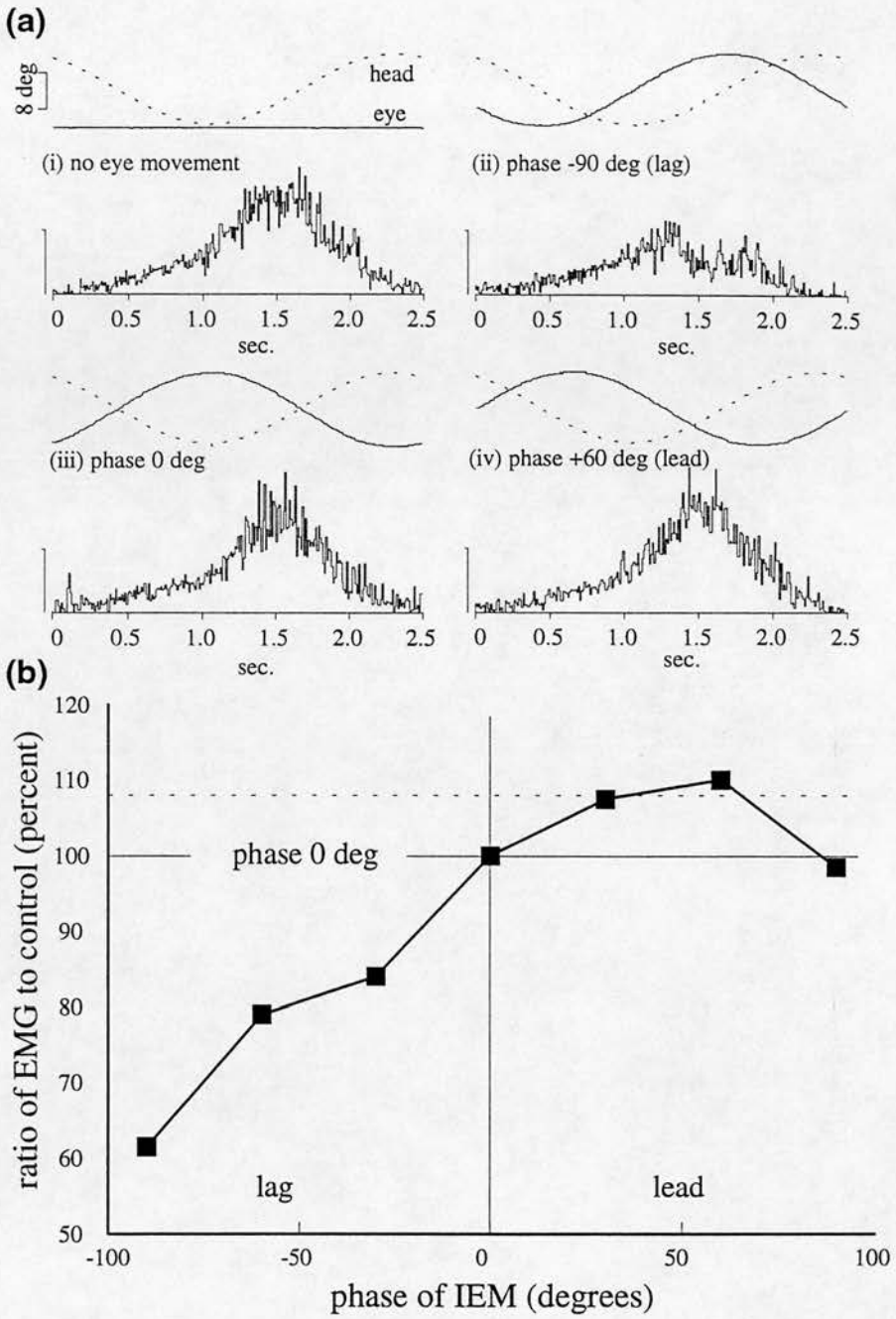


Figure 2.23. Effect of varying the phase of the 'artificial VOR' (aVOR) on VCR response of the left *splenius*. (a) Four cycle histograms (CHSTs) from an interleaved set of eight, showing the averaged electromyographic (EMG) activity during the VCR response of the left *splenius*; (i) no aVOR; (ii) aVOR at compensatory velocity, but with a phase lag of 90°; (iii) aVOR at compensatory velocity and phase (compensatory aVOR) and (iv) aVOR at compensatory velocity for vestibular stimulus, but with a phase lead of 60°. Broken line shows head position, solid line shows eye position. Scale bars 2.56 μ V. (b) Results from the same experiment as (a). Plot of the ratio of the VCR response of the left *splenius* during imposed movements of the left eye with various phases (± 90°), to its VCR response at the compensatory aVOR. The VCR response is decreased with increasing phase lag, and increased with increasing phase lead. The broken line is the VCR response when there was no eye movement.

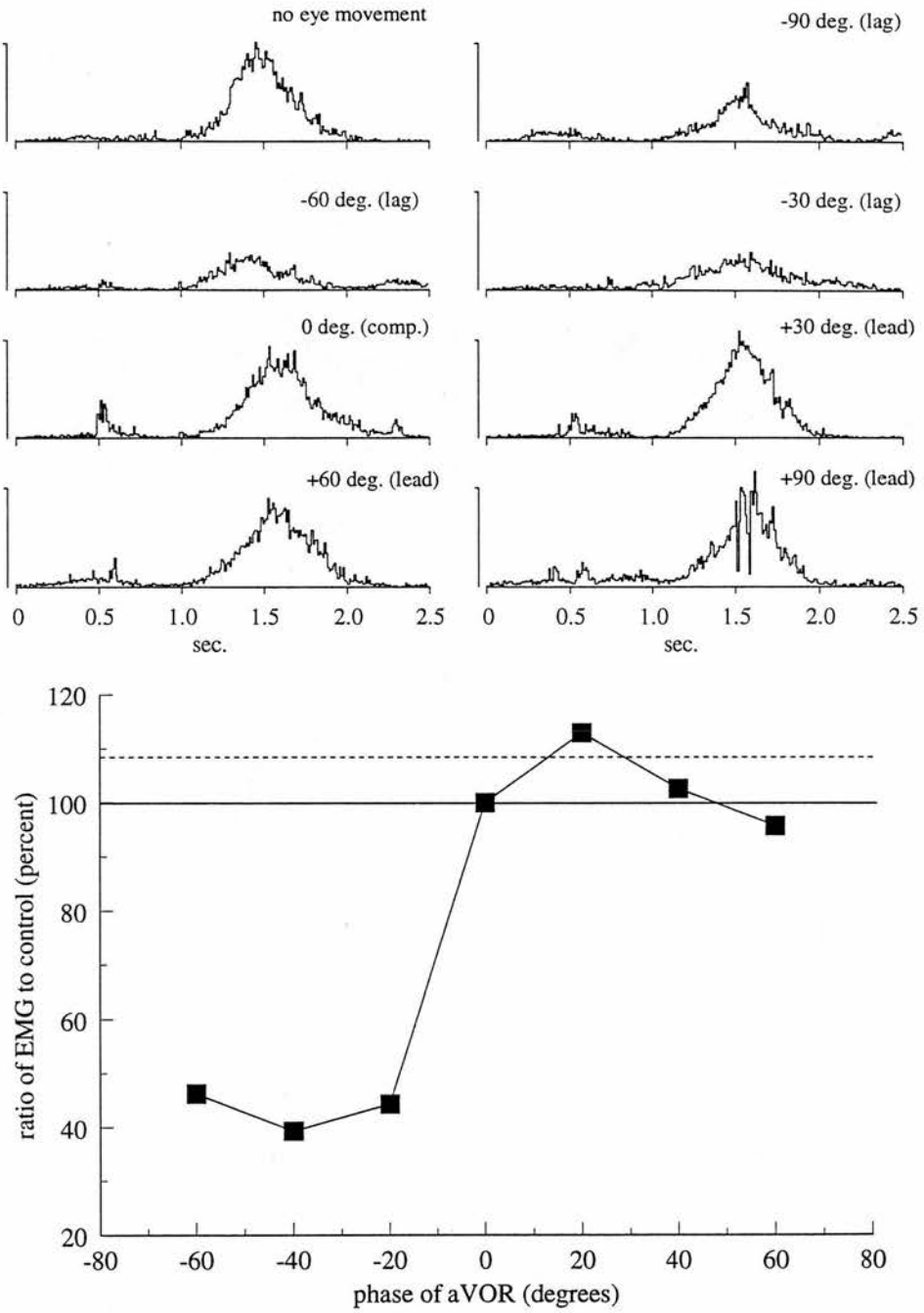


Figure 2.24. Set of eight interleaved cycle histograms (CHSTs) from an experiment investigating the effect of the artificial VOR (aVOR) with phase errors on the left *complexus*. The peak imposed eye velocity was always 22°s^{-1}, thus producing an aVOR compensatory in speed for the head movement, and the phase of the eye movement was varied from 90° phase lead (+90°) to 90° phase lag (-90°) relative to the compensatory aVOR (compensatory both in speed and phase, taken as phase 0°). Phase lags resulted in reductions in the VCR response compared to that of the compensatory VOR and phase leads produced slight increases. Solid line is VCR response at compensatory aVOR, broken line is VCR response when the eye was held still. Scale bars for CHSTs 6.4 μV.

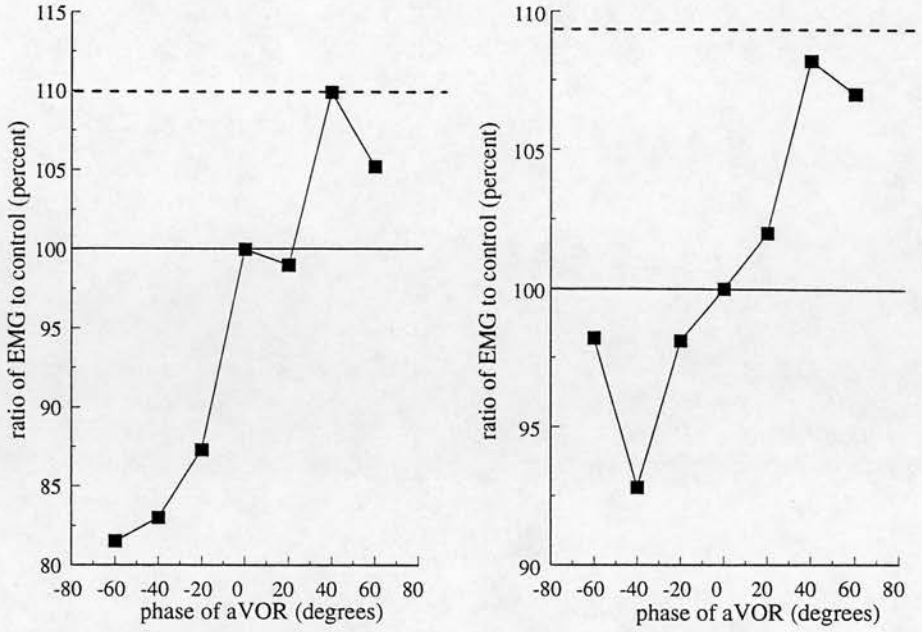


Figure 2.25. Effects of artificial VOR (aVOR) with phase errors on the right *complexus* (left-hand side) and *splenius* (right-hand side). Graphs derived as described in Figure 2.23. The effect of phase leads and lags is similar to that seen in the ipsilateral muscles, but the magnitudes of the decreases/increases are much smaller.

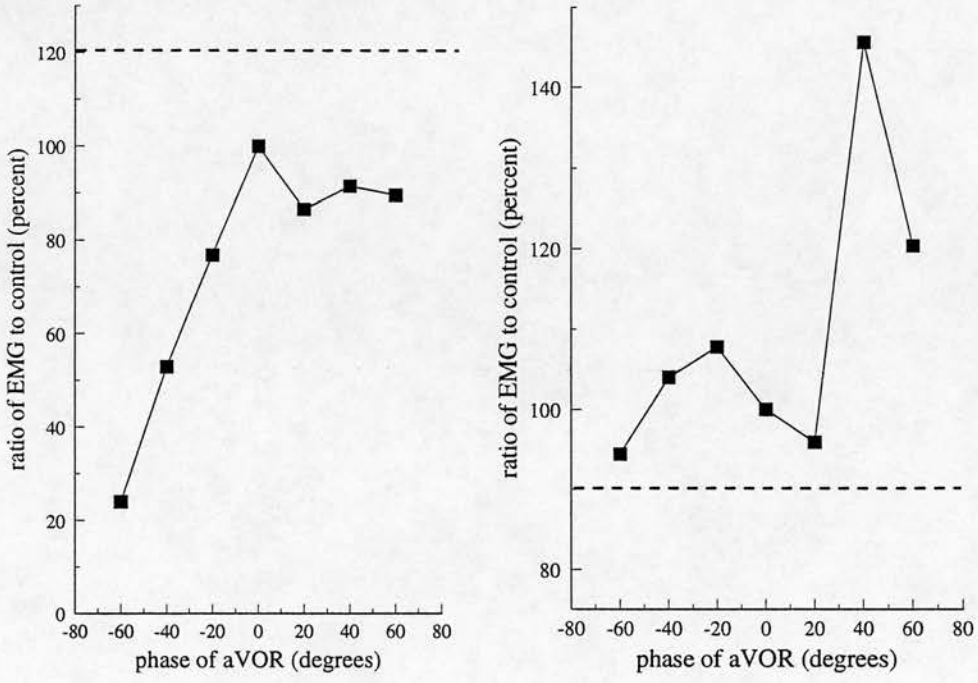


Figure 2.26. Effect of artificial VOR (aVOR) with phase errors on the VCR response of the left (left-hand side) and right (right-hand side) *rectus capitis ventralis lateralis* (*r.c.v.l.*). Plots are derived from sets of eight interleaved cycle histograms as described in Figure 2.23. An aVOR with phase errors inhibits the VCR response of the left *r.c.v.l.* below that seen at the compensatory aVOR (phase 0°). An aVOR with phase errors produces an increase in the VCR response of the right *r.c.v.l.* The VCR response of the left *r.c.v.l.* is always below that seen when the eye was held still, whereas the VCR response of the right *r.c.v.l.* is always above the VCR response when the eye was held still.

contralateral muscle, something that was not seen in the contralateral *splenius* or *complexus*.

As was found for the aVOR with amplitude/velocity errors the effect of the aVOR with phase errors was always on the gain or magnitude of the VCR response of a particular muscle and never on the phase of the response, which remained approximately constant, as can be seen in Figure 2.22.

Responses to natural vestibular stimulation in the frontal plane (roll tilt)

Effect of sIEM

As mentioned earlier, all four of the muscles studied, *splenius*, *complexus*, *rectus capitis ventralis lateralis* and *biventer cervicis*, responded with vestibularly-evoked activity to vestibular stimulation in the frontal plane. Due to the limitations of the eye-mover used in this series of experiments, tests of directional 'tuning' were not possible. The eye-mover was restricted to movements in the vertical plane (0° and 180°) only. Experiments studying the effect of amplitude directed vertically upwards (0°) and downwards (180°) did demonstrate differences in the sensitivity of muscles to the direction of sIEM, however. Figure 2.27 shows an example of the effect of increasing amplitudes (5° to 20°) of sIEM in the vertical plane on the vestibular response of the right *complexus*; the right eye was being moved. Increasing amplitudes of sIEM directed vertically upwards (0°) produced large and sustained inhibitions in the vestibular response. sIEM directed vertically downwards (180°) inhibited the vestibular response of the right *complexus* to a lesser extent. Such directionally-specific effects were seen in 10 of 13 experiments on the right *complexus*. A very similar effect was seen for the right *rectus capitis ventralis lateralis* (Figure 2.28), sIEM directed vertically upwards completely abolishing the vestibular response of the muscle, with sIEM directed vertically downwards producing a marked but lesser inhibition.

The vestibular activity of the right *splenius* was also inhibited by vertically directed sIEM during roll tilt as shown in Figure 2.28, but this inhibition was affected only by the amplitude of the sIEM and not the direction; this was seen in 6 out of 6 experiments on the right *splenius*. The type of inhibition seen in *splenius* was different from that seen in *complexus* or *rectus capitis ventralis lateralis*. The initial phasic portion of the imposed trapezoid produced an inhibition in the

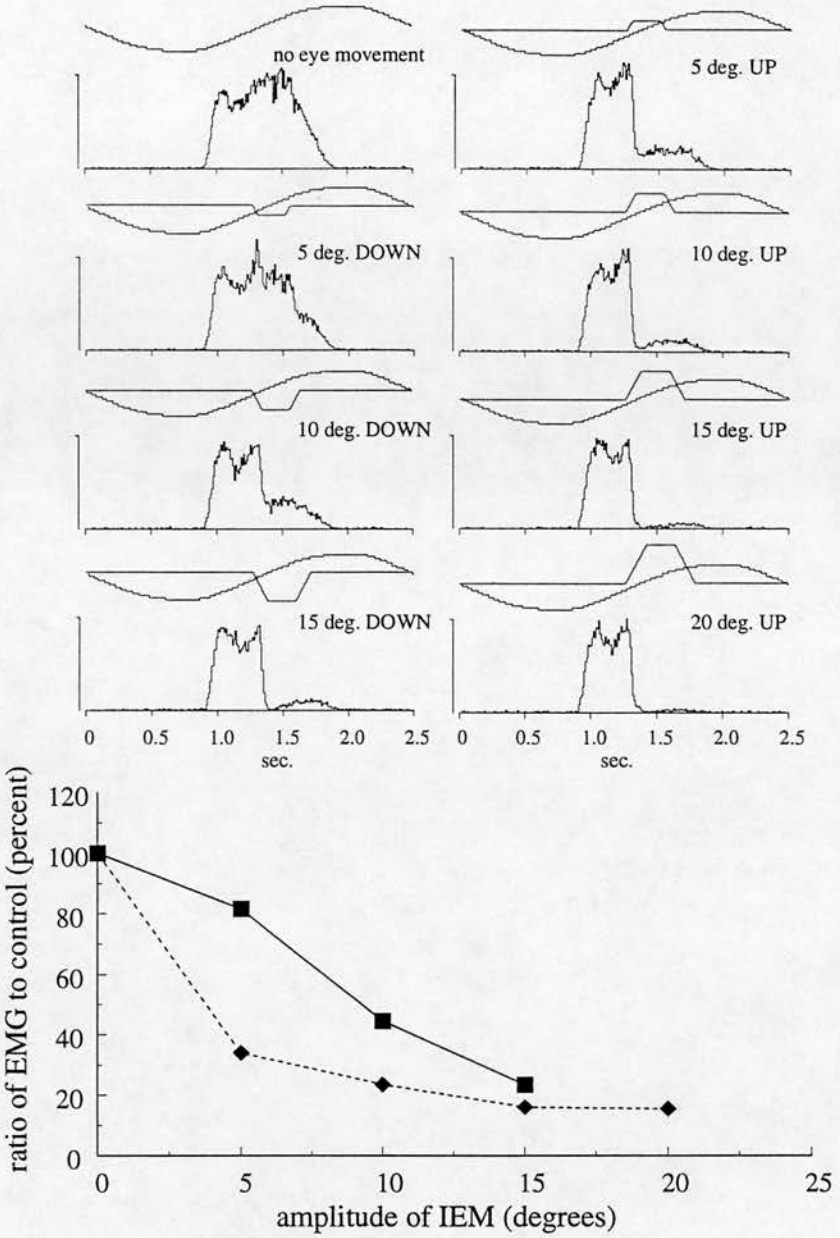


Figure 2.27. Set of eight interleaved cycle histograms (CHSTs) showing the effect of changes in the amplitude of saccadic imposed eye movement (sIEM) of the right eye directed vertically upwards (trapezoid directed upwards) and downwards (trapezoid directed downwards) on the electromyographic activity of the right *complexus* during frontal, sinusoidal vestibular stimulation (VEST) (± 8° at 0.4 Hz). Each CHST represents exactly one cycle of vestibular oscillation. Upward deflection of vestibular table position trace (solid sinusoid) represents rotation to the left (right ear upwards). Eye position trace (solid line containing trapezoid) shows the time course of sIEM. The top left CHST shows the response to VEST alone (eye held still). The remaining CHSTs show the VCR response to VEST and added sIEM of various amplitudes (5° to 20°, vertically upwards and 5° to 15°, vertically downwards). The graph in the lower part of the figure shows the ratio of the modulation of the averaged EMG activity during combined VEST and sIEM, to the modulation of the EMG activity during VEST alone (control), plotted against the amplitude of the sIEM (diamonds, sIEM directed vertically upwards; squares, sIEM directed vertically downwards). The magnitude of the reduction in EMG activity is dependent on the direction of the eye movement, as well as on its amplitude, with movements vertically upwards having the largest effect; these differences are statistically significant ($P<0.005$). Scale bars for CHSTs 160 μV.

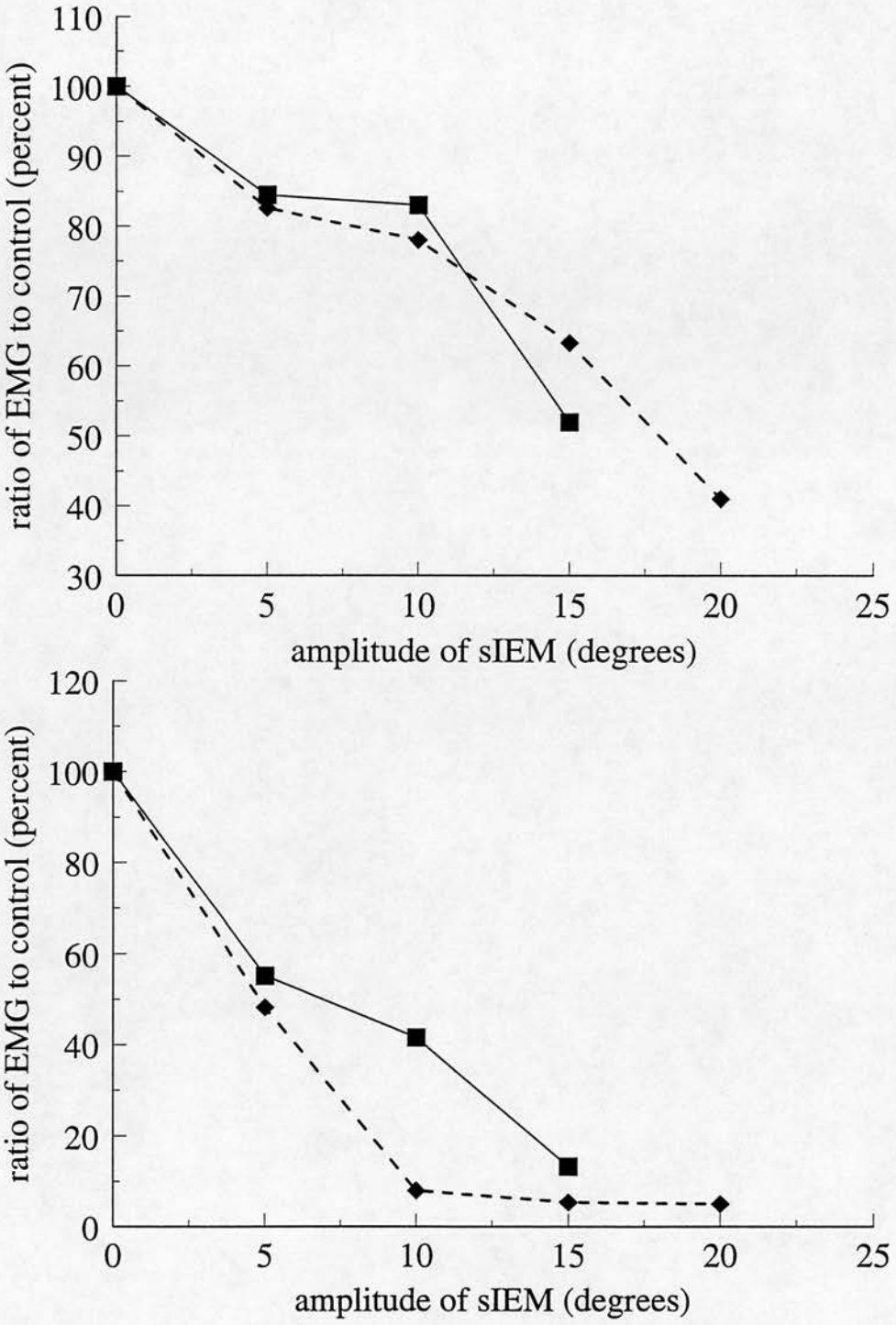


Figure 2.28. Effect of changes in the amplitude of saccadic imposed eye movement (sIEM) of the right eye directed vertically upwards (diamonds) and downwards (squares) on the VCR response of the right *splenius* (top graph) and *rectus capitis ventralis lateralis* (*r.c.v.l.*) (bottom graph). The graph is constructed as described in Figure 2.27. The magnitude of the reduction in the VCR response is dependent on the direction of sIEM as well as on its amplitude. The effect of sIEM on *r.c.v.l.* depends on the direction of eye movement as well as on its amplitude with sIEM directed vertically upwards producing consistently larger inhibitions in the VCR response ($P<0.005$, for all amplitudes). The direction of sIEM does not have a consistently different effect on the inhibition of the VCR response of the right *splenius.*

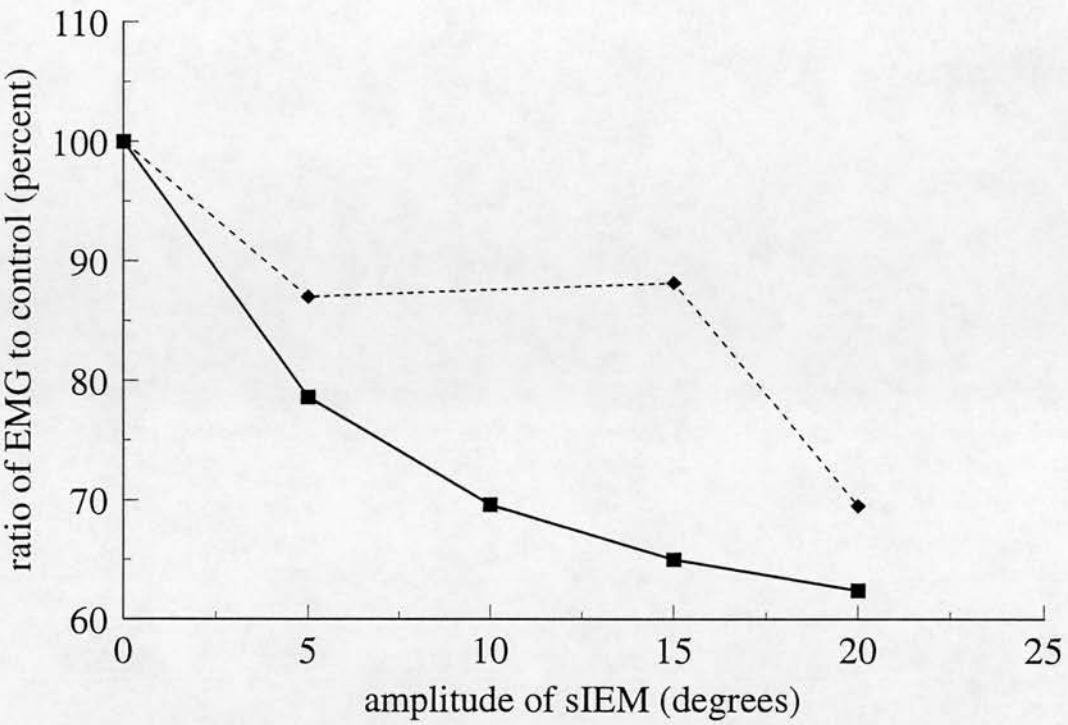


Figure 2.29. Effect of changes in the amplitude of saccadic imposed eye movement (sIEM) of the right eye on the VCR response of the left *complexus* during frontal vestibular stimulation (VEST) directed vertically upwards (diamonds) or downwards (squares). Graph is constructed in exactly the same manner as that in Figure 2.27. sIEM directed vertically downwards produces considerably larger inhibitions than sIEM directed vertically upwards. The differences between the inhibition of the VCR response for sIEM directed vertically upwards and downwards are statistically significant ($P<0.001$) for each of the amplitudes tested.

vestibular activity, but this inhibition disappeared during the S2 section of sIEM when the eye was held eccentrically in the orbit. This type of phasic inhibition of *splenius* and longer lasting inhibition of *complexus* and *rectus capitis ventralis lateralis* was also seen with sIEM during VEST in the horizontal plane.

The effects of sIEM on the contralateral (left) *complexus* during roll tilt also showed directional tuning. sIEM inhibited the vestibular activity in this muscle, with imposed movements initially directed vertically downwards producing a larger inhibition than those directed vertically upwards (see Figure 2.29). As was seen in during VEST in the horizontal plane, the contralateral muscle is affected to the greatest extent by sIEM directed in the opposite direction to that which produces the greatest inhibition in the ipsilateral muscle.

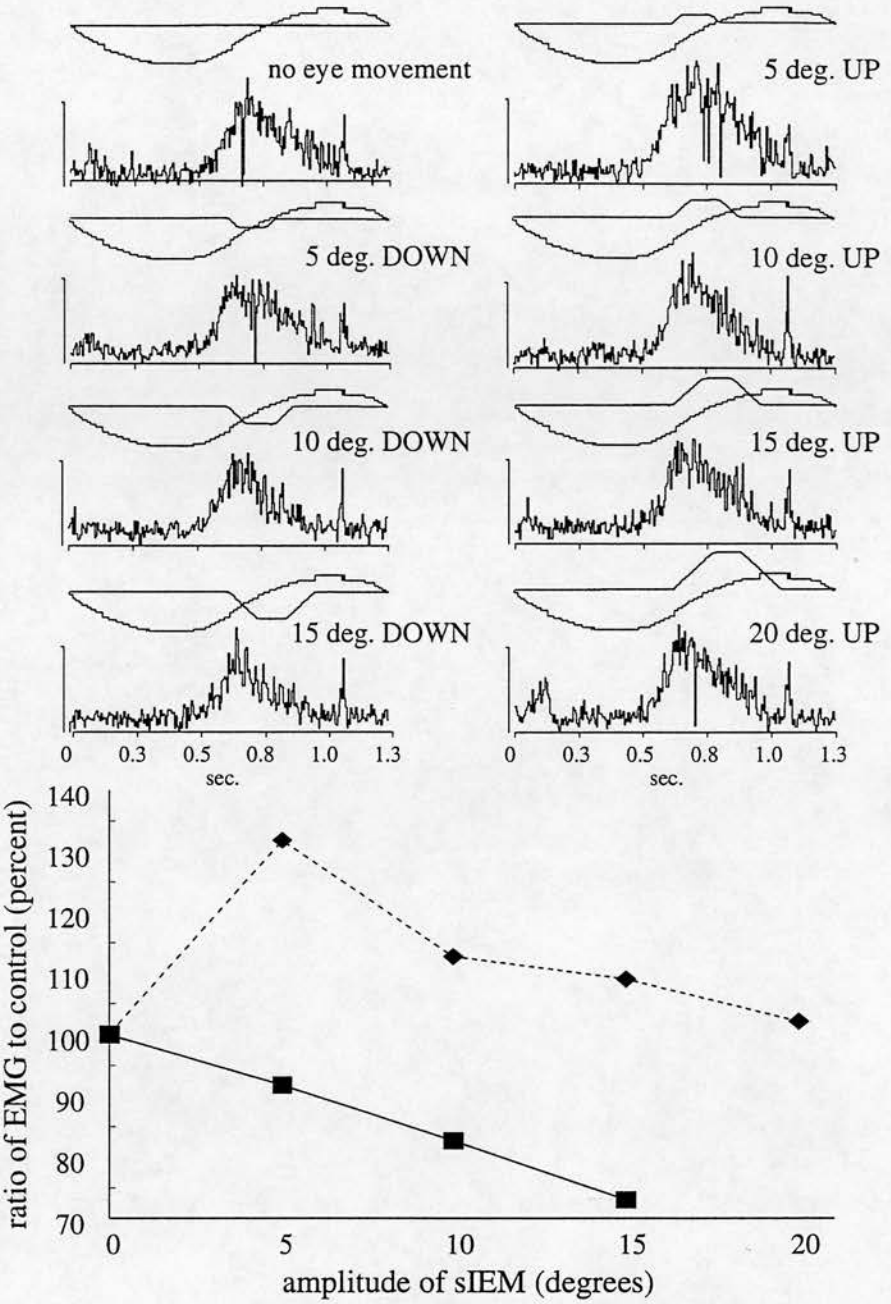


Figure 2.30. Set of eight interleaved cycle histograms (CHSTs) showing the effect of changes in the amplitude of saccadic imposed eye movement (sIEM) of the right eye directed vertically upwards (trapezoid directed upwards) and downwards (trapezoid directed downwards) on the electromyographic activity of the right *biventer cervicis* during frontal, sinusoidal vestibular stimulation (VEST). Methods of stimulation are as described in Figure 2.27. The graph in the lower part of the Figure is constructed as explained in the legend to Figure 2.27. The magnitude of the modulation of electromyographic activity is dependent on the direction of saccadic imposed eye movement (sIEM). While increasing amplitude produces inhibition in the VCR response of *biventer cervicis*, sIEM directed vertically upwards (diamonds) increases the VCR response above that seen when the eye was held still (top left CHST, control), whereas it is consistently inhibited below the control level by sIEM directed vertically downwards (squares). The differences between the magnitude of the inhibition of the VCR response produced by sIEM directed vertically upwards and downwards are statistically significant ($P<0.001$). Scale bars for CHSTs 6.4 μV.

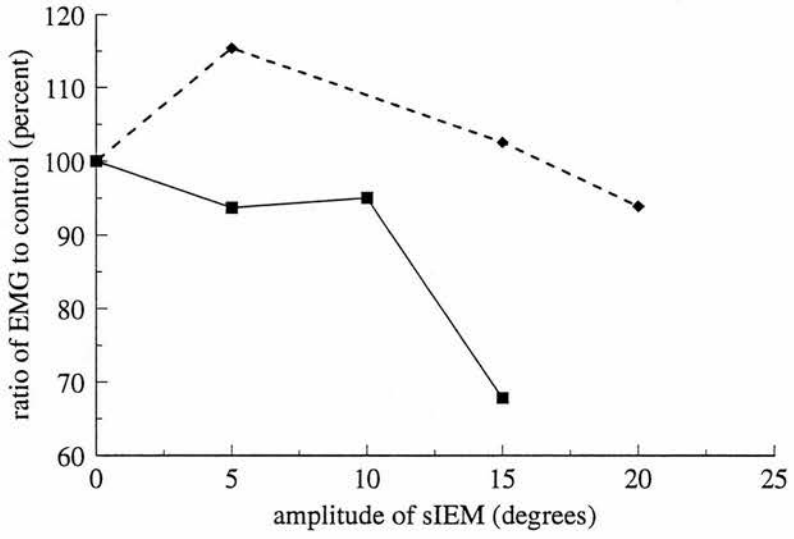


Figure 2.31. Effect of changes in the amplitude of saccadic imposed eye movement (sIEM) of the right eye on the VCR response of the left *biventer cervicis* during frontal vestibular stimulation (VEST) directed vertically upwards (diamonds) or downwards (squares). Graph is constructed in exactly the same manner as that in Figure 2.27. While increasing amplitude produces inhibition in the VCR response of the left *biventer cervicis*, sIEM directed vertically upwards (diamonds) increases the VCR response above that seen when the eye was held still (top left CHST, control), whereas it is consistently inhibited below the control level by sIEM directed vertically downwards (squares). The differences between the magnitude of the inhibition of the VCR response produced by sIEM directed vertically upwards and downwards are statistically significant ($P<0.001$).

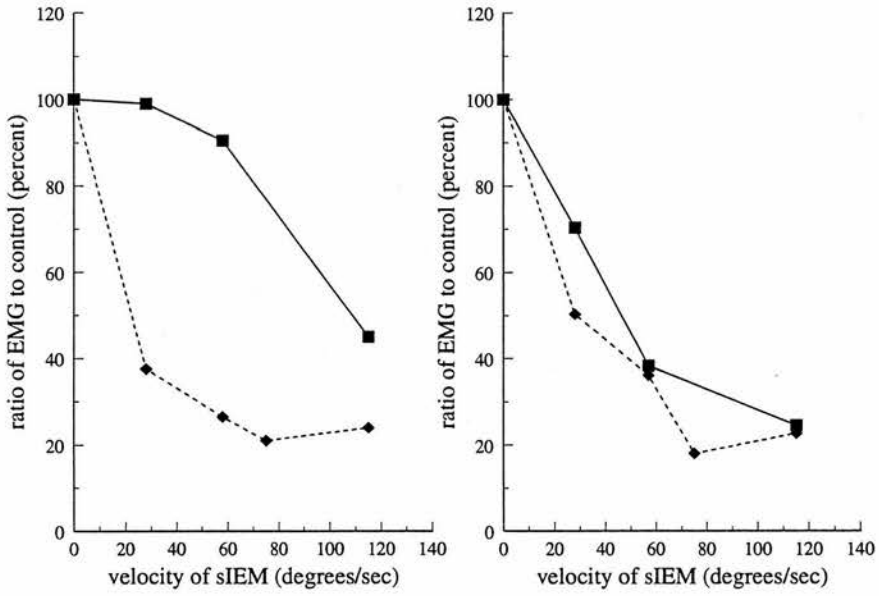


Figure 2.32. Effect of change of velocity ($28°s^{-1}$ to $115°s^{-1}$) of vertical saccadic imposed eye movement (sIEM) of the right eye directed vertically upwards (diamonds) or downwards (squares), amplitude held constant at 15°, on the right *complexus* (left-hand side) or right *splenius* (right-hand side). The graph is constructed in exactly the same manner as that of Figure 2.19. The magnitude of the reduction in electromyographic activity is dependent on the direction of sIEM for *complexus* as well as the velocity, but there is a far smaller variation in the effect of the two directions of sIEM for *splenius*.

The right *biventer cervicis* was inhibited by sIEM directed vertically downwards during roll tilt and was further inhibited by increasing the amplitude of the sIEM. Unlike the other muscles studied during vestibular stimulation in the horizontal or frontal planes, the vestibular activity of *biventer cervicis* was increased by sIEM directed vertically upwards. The magnitude of this increase was reduced by increasing amplitudes of sIEM (see Figure 2.30). This was seen in 3 out of 4 experiments on *biventer cervicis* during roll tilt. The VCR response of the contralateral (left) *biventer cervicis* showed the same directional tuning as the ipsilateral muscle, something that was not seen for any other contralateral muscle during frontal or horizontal VEST. sIEM directed vertically upwards increased the VCR response of the left biventer cervicis and sIEM directed vertically downwards decreased the VCR response of the muscle (Figure 2.31).

Vestibular activity in all of the muscles studied was also affected by the velocity of the sIEM. As can be seen in Figure 2.32, the direction of the sIEM also affected the magnitude of the inhibition of the VCR response for the right *complexus*, but not for the right *splenius*, in agreement with the effects of direction and amplitude of sIEM on these muscles.

Effects of the artificial VOR

As was seen during vestibular stimulation in the horizontal plane, the artificial VOR produced long lasting inhibition of the vestibular activity in all of the muscles studied during the whole of the vestibular response of these muscles. Likewise the phase of the vestibular response of all the muscles tested remained virtually unchanged during the aVOR at any velocity or phase; all effects produced by the aVOR were on the gain of a muscle's vestibular response.

Imposing velocity/amplitude errors with the aVOR produced systematic changes in the vestibular response of *complexus*, *rectus capitis ventralis lateralis* and *splenius* that were very similar to those seen during horizontal vestibular stimulation. Again comparing the effects with those seen when imposing an aVOR at the compensatory velocity, an aVOR slower than compensatory increased the vestibular response of a muscle while an aVOR with a faster speed than required for compensation reduced a muscle's vestibular response. As was done with the results in the horizontal plane, the results of each experiment imposing the aVOR with amplitude/velocity errors in the compensatory direction were collated. All of the

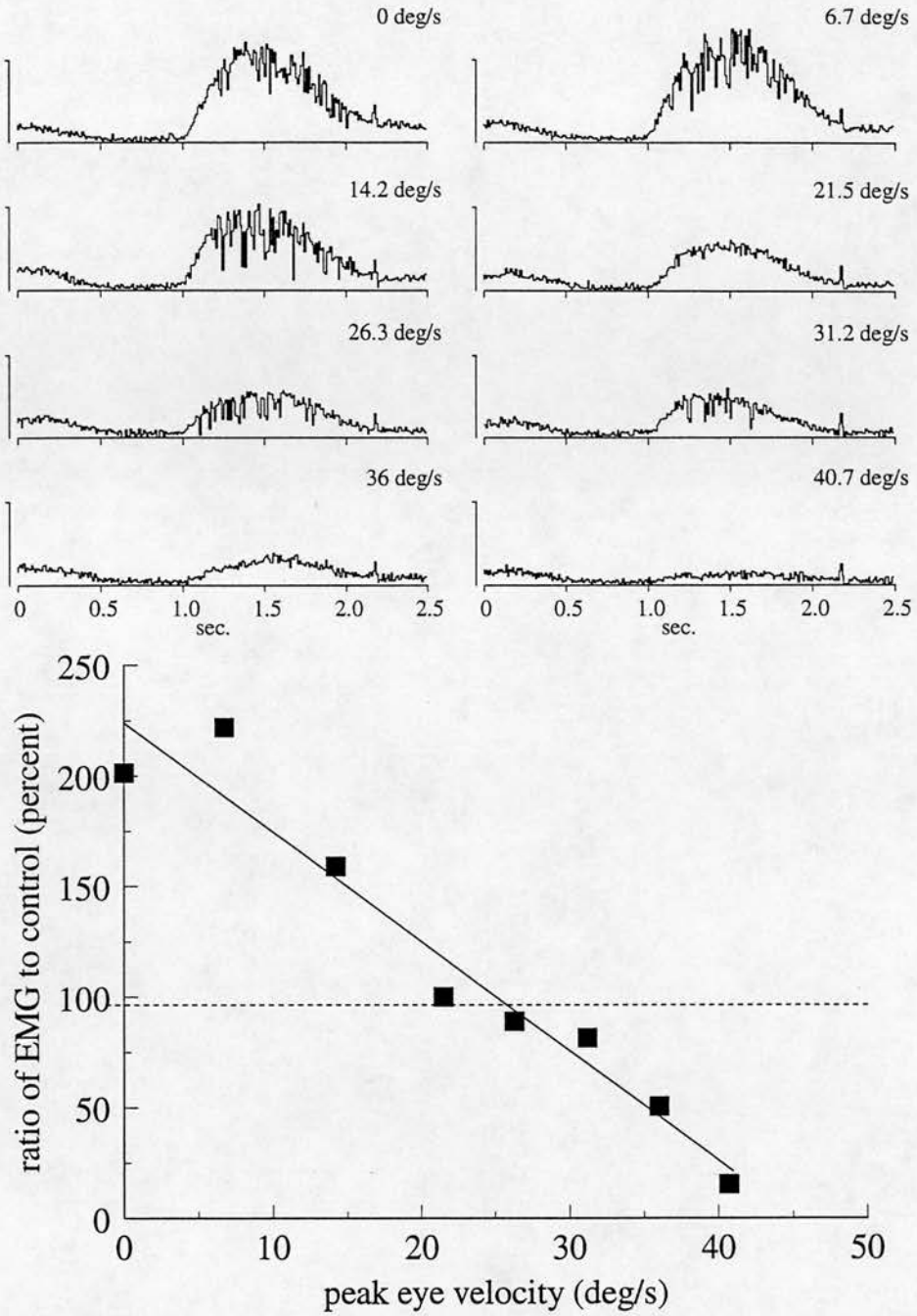


Figure 2.33. Set of eight interleaved cycle histograms (CHSTs) showing the VCR response of the right *splenius* during sinusoidal, frontal vestibular stimulation (VEST) (± 8° at 0.4 Hz) alone (top left CHST) and during the artificial VOR (aVOR) with the peak eye velocities marked against the CHSTs. The response at 21.5°s^{-1} represents that during the compensatory aVOR when the head and eye speed are equal but in the opposite direction. The VCR response is greater than that at the compensatory aVOR when peak speeds slower than compensatory are imposed and smaller when higher speeds are imposed. The graph in the lower part of the figure is derived in the same manner as that of Figure 2.20. The solid line is the linear regression ($r = 0.97$, $P<0.001$). The slope of the regression line is -4.9, suggesting that the VCR response falls by about 5% for each 1°s^{-1} increase in peak eye velocity. The dotted line shows the VCR response at the compensatory velocity (21.5°s^{-1}). Scale bars for CHSTs 6.4 μV.

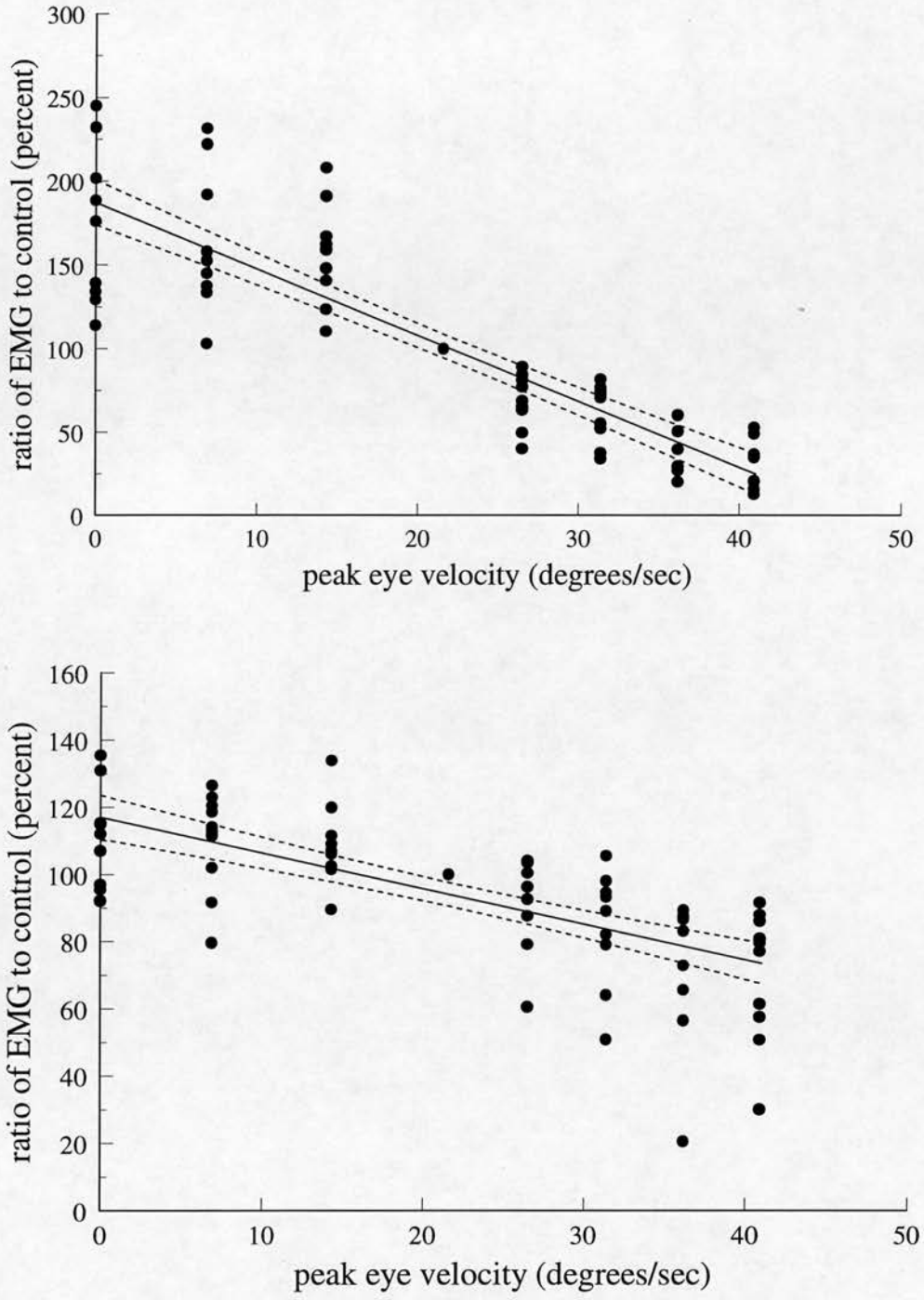


Figure 2.34. Linear regressions of the: (ratio of the response at various velocities of aVOR to control response, the compensatory aVOR) to (peak eye velocity). Top graph shows the linear regression for 70 pairs of observations from 9 experiments on the right *complexus* or *splenius*. The correlation coefficient is highly significant ($r = 0.88$; $P<0.001$). The VCR response falls, on average, by about 4% for each $1°s^{-1}$ increase in the peak eye velocity during the aVOR. Bottom graph shows the linear regression for 80 pairs of observations from 10 experiments on the right *complexus*, *splenius* or *rectus capitis ventralis lateralis*. The correlation coefficient is highly significant ($r = 0.69$; $P<0.001$). The VCR response falls, on average, by about 1% for each $1°s^{-1}$ increase in the peak eye velocity during the aVOR. Dotted hyperbolas show 95% confidence limits for regression lines.

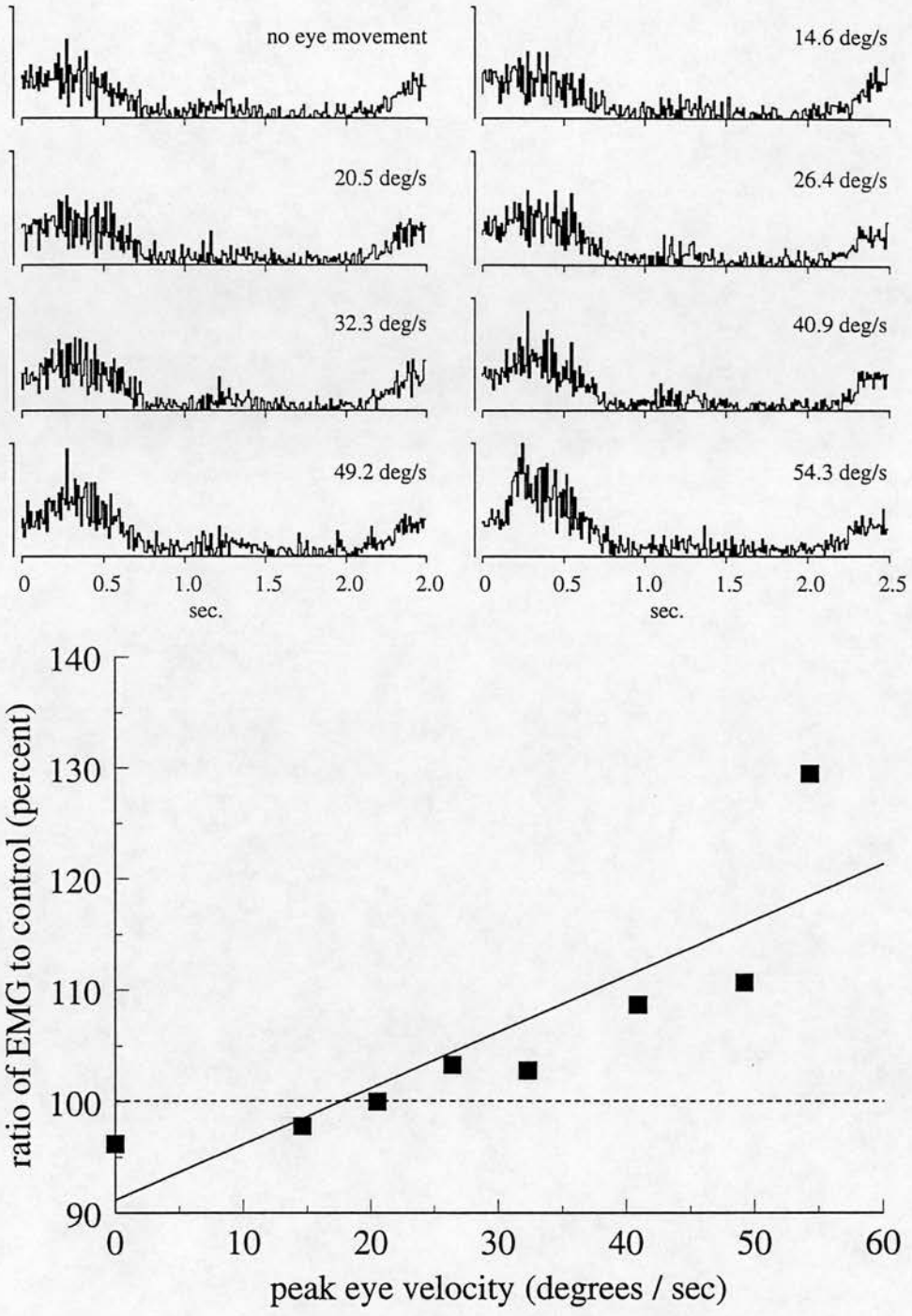


Figure 2.35. Set of eight interleaved cycle histograms (CHSTs) showing the VCR response of the left *biventer cervicis* during sinusoidal, frontal vestibular stimulation (VEST) (± 8° at 0.4 Hz) alone (top left CHST) and during the artificial VOR (aVOR) with the peak eye velocities marked against the CHSTs. The response at 20.5°s^{-1} represents that during the compensatory aVOR when the head and eye speed are equal but in the opposite direction. The VCR response is greater than that at the compensatory aVOR when peak speeds **larger** than compensatory are imposed and **smaller** when lower speeds are imposed. The graph in the lower part of the figure is derived in the same manner as that of Figure 2.20. The solid line is the linear regression ($r = 0.86$, $P<0.001$). The slope of the regression line is 0.50, suggesting that the VCR response increases by about ½% for each 1°s^{-1} increase in peak eye velocity. The dotted line shows the VCR response at the compensatory velocity. Scale bars for CHSTs 6.4μV.

experiments performed (19) had correlation coefficients that were greater than r=0.7 ($P<0.05$) when linear regressions were constructed of : (ratio of responses at various velocities of aVOR to control response, the compensatory aVOR) to (velocity of aVOR). Analysis of covariance of the regression data for pairs of experiments from a particular muscle with the widest spread of parameters and/or largest difference in regression slopes suggested that the regression lines formed two distinct groups, each homogeneous in slope, one with a mean slope of approximately -1 and the other with a mean slope of approximately -4. Figure 2.33 shows an example of the effect of the aVOR with amplitude/velocity errors on the right *splenius*. The data from the individual muscles were pooled into two groups depending on their regression slopes. Combining the data from 9 experiments produced the regression plotted in the upper half of Figure 2.34 which has a slope of -3.96. The correlation coefficient (r) of 0.88 for 68 degrees of freedom gives $P<0.001$ which is highly statistically significant. The combined regression slope means that for each degree-per-second increase in velocity of eye movement during the aVOR there was a reduction of about 4% in the vestibular response of a subset of *splenius* and *complexus*. Combining the data from 10 experiments produced the regression plotted in the lower half of Figure 2.34 which has a slope of -1.06. The correlation coefficient (r) of 0.69 for 78 degrees of freedom gives $P<0.001$. The combined regression slope of -1.06 means that for each degree-per-second increase in the velocity of imposed eye movement there was a reduction of about 1% in the vestibular response of a subset of *complexus* and *splenius* and all of the *rectus capitis ventralis lateralis* muscles studied.

The effect of the aVOR with velocity errors on *biventer cervicis* was strikingly different from that seen in the other muscles studied with horizontal or frontal vestibular stimulation. The ipsilateral muscle showed similar inhibitions in its VCR response with the aVOR directed in the compensatory direction to the three other muscles studied. However, the effect of the aVOR with amplitude/velocity errors on the contralateral muscle's vestibular response was opposite to that seen in any other contralateral or ipsilateral muscle. Increasing the velocity of the aVOR above the compensatory velocity increased the contralateral *biventer cervicis'* VCR response, whereas decreasing the velocity of the aVOR below that of the compensatory aVOR reduced its vestibular activity (Figure 2.35). The vestibular response of the contralateral muscle during vestibular stimulation alone was smaller than the activity during the aVOR with the lowest velocity.

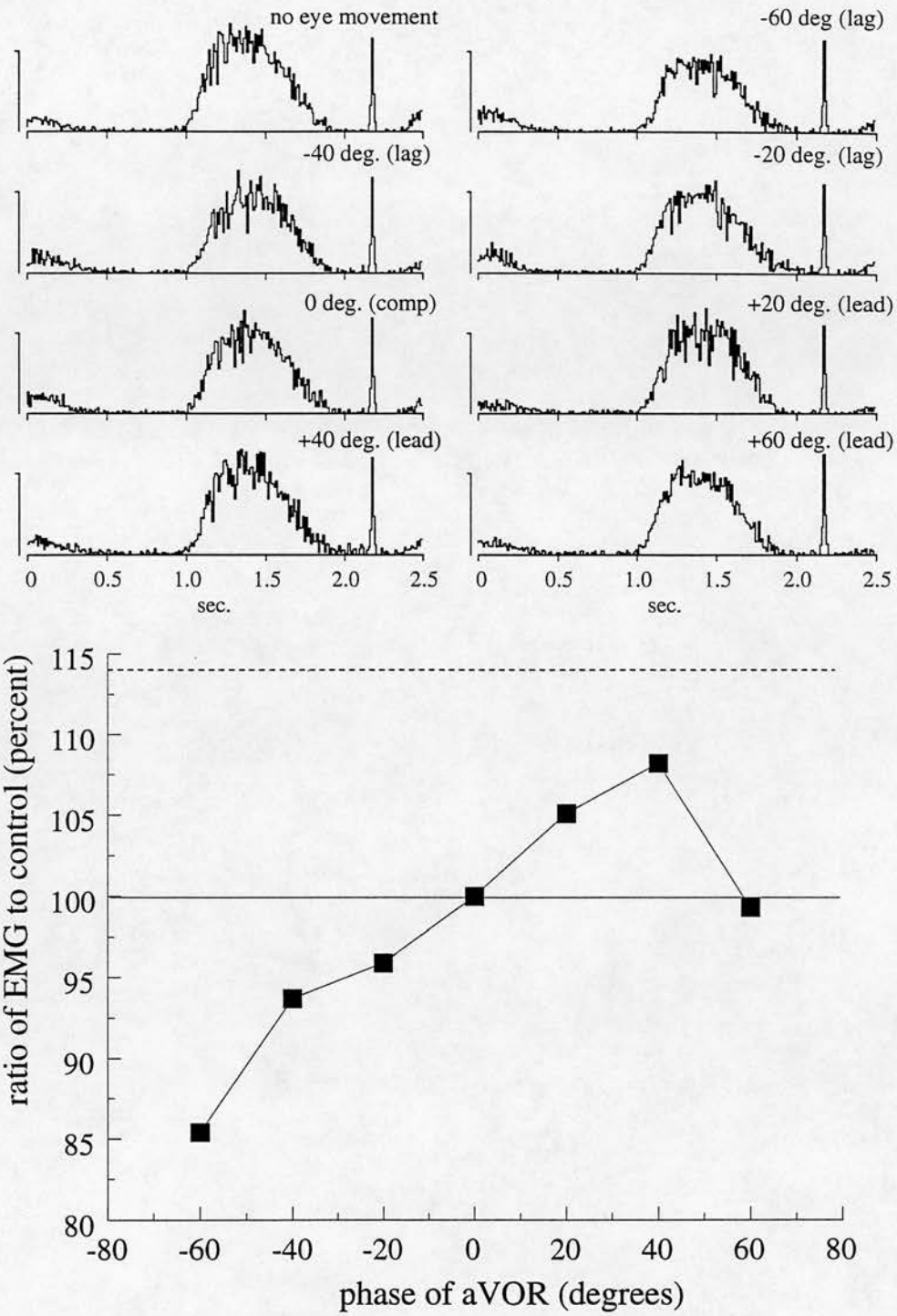


Figure 2.36. Set of eight interleaved cycle histograms (CHSTs) investigating the effect of the artificial VOR (aVOR) with phase errors on the right *complexus* during frontal vestibular stimulation (VEST). Methods of stimulation are as described in Figure 2.23. Scale bars for CHSTs, 12.5 μV. The graph in the lower part of the figure is derived in the same manner as that of Figure 2.23. An aVOR with a phase lag inhibits the VCR response below that seen at the compensatory aVOR (phase 0°), whereas an aVOR with a phase lead increases the VCR response above that seen at the compensatory aVOR. Solid line shows the VCR response at the compensatory aVOR, dotted line shows the VCR response when the eye was held still.

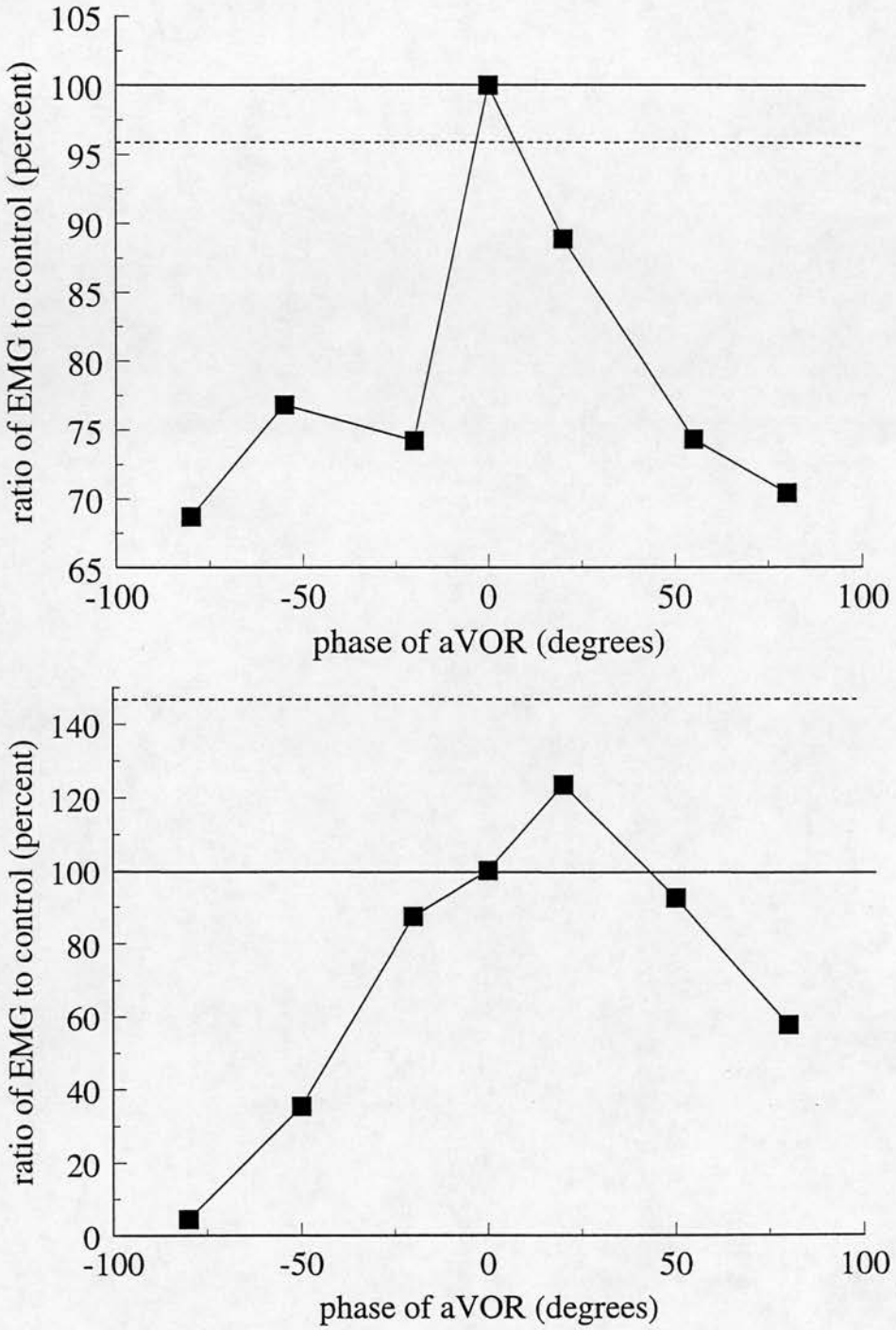


Figure 2.37. Effect of artificial VOR (aVOR) with phase errors on the VCR response of the right *splenius* (top graph) and *rectus capitis ventralis lateralis* (*r.c.v.l.*) (bottom graph). Graphs are constructed as described in Figure 2.23. Phase lags inhibit the VCR response of both the right *splenius* and *r.c.v.l.* below that seen at the compensatory aVOR (phase 0°). Phase leads cause a similar decrease in the VCR response of *splenius*, but produce a slight increase in the VCR response of *r.c.v.l.* Solid line shows VCR response at compensatory aVOR, dotted line shows VCR response when the eye was held still.

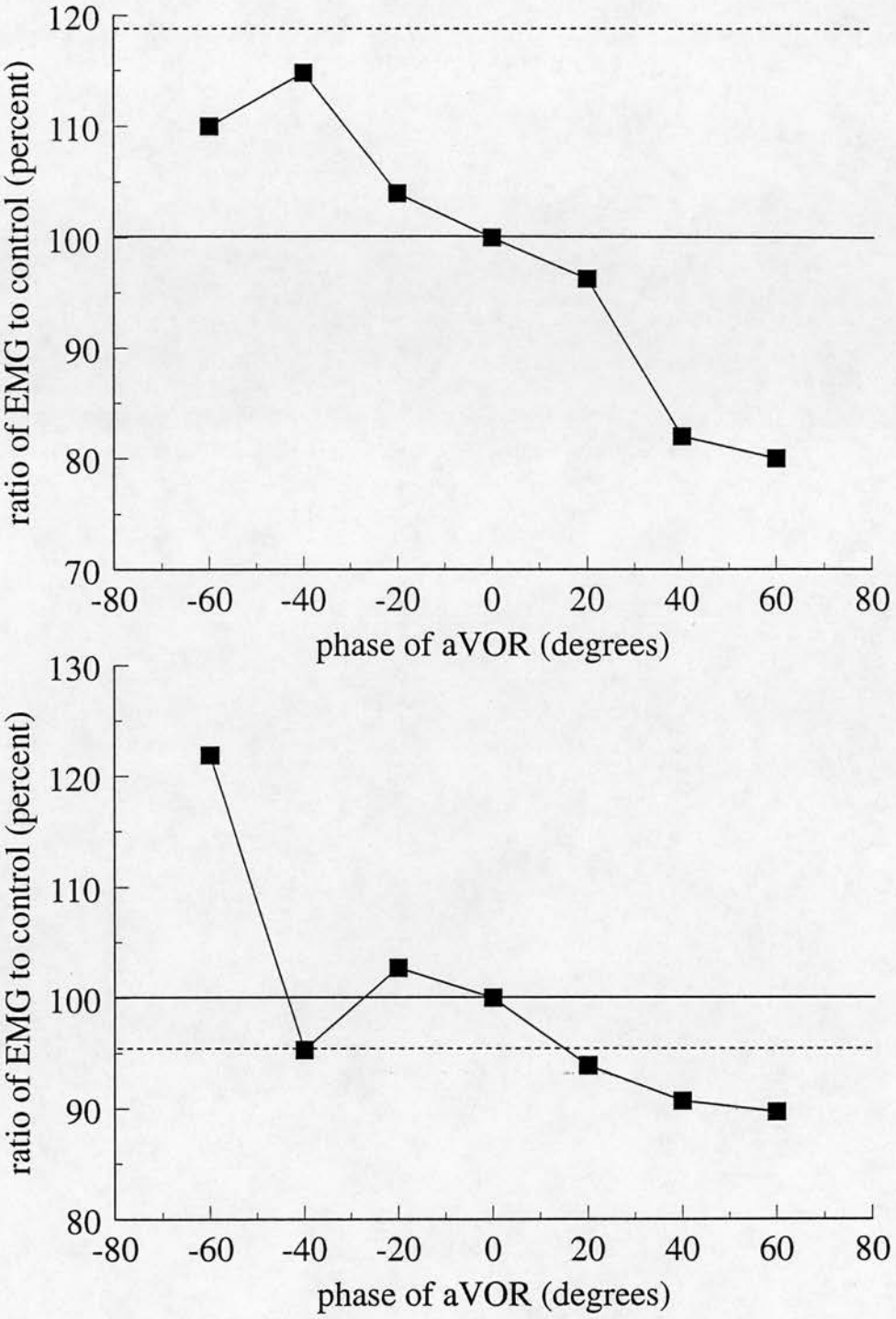


Figure 2.38. Effect of artificial VOR (aVOR) with phase errors on the VCR response of the right *biventer cervicis* (top graph) and left *biventer cervicis* (bottom graph). Graphs are constructed as described in Figure 2.23. Phase lags increase the VCR response of the right (ipsilateral) and left (contralateral) *biventer cervicis* above that seen at the compensatory aVOR (phase 0°). Phase leads cause a decrease in the VCR response of both muscles. Solid lines show VCR response at compensatory aVOR, dotted lines show VCR response when the eye was held still.

Altering the phase of the aVOR whilst maintaining the amplitude and velocity of IEM at that of the compensatory aVOR produced systematic changes in the vestibular response of *complexus* and *rectus capitis ventralis lateralis*. Increasing phase lags produced increasing inhibitions in the VCR response, with increasing phase leads producing slight increases in the VCR response compared to that at the compensatory aVOR (Figure 2.36 & 2.37). In *splenius* imposing an aVOR with a phase lag or phase lead inhibited the muscle's vestibular response compared to the VCR response at the compensatory aVOR (phase 0°) to a similar extent (Figure 2.37). *Biventer cervicis* again showed notable differences to the effects seen in the other muscles, phase lags increasing the VCR response above the VCR response seen at the compensatory aVOR (phase 0°) and phase leads decreasing the VCR response in both the ipsilateral and contralateral muscles (Figure 2.38).

Effects of imposed eye movement during rotation in the sagittal plane (pitch)

A few experiments were carried out to study the effect of vertical imposed eye movements during pitch. While natural eye movements would be torsional during vestibular stimulation in the sagittal plane, the limitations of the eye mover meant that only vertical eye movements (again at 0° and 180°) could be imposed.

sIEM directed vertically upwards or downwards inhibited the vestibular response of *complexus* and *biventer cervicis*, the only two muscles to be tested in this plane. *Complexus* was inhibited to the greatest extent by sIEM directed vertically downwards, showing long lasting inhibition to the initial, S1, portion of the imposed trapezoid with inhibition beginning approximately 50 milliseconds (5×10ms bin widths) after the start of sIEM in a similar manner to that seen during horizontal and frontal vestibular stimulation. *Biventer cervicis* was also most inhibited by eye movements directed vertically downwards, but this inhibition was not as long lasting as that seen in *complexus*. The inhibition produced by sIEM did not appear until the eye was being held eccentrically, the S2 portion of the imposed trapezoid, with a latency of approximately 10 milliseconds after the start of the S2 portion of sIEM. Activity recovered in the muscle during the centripetally directed final portion, S3, of the imposed trapezoid with a latency of approximately 70 milliseconds. The magnitude of the inhibition was also dependent on the amplitude of the imposed eye movement (see Figure 2.39).

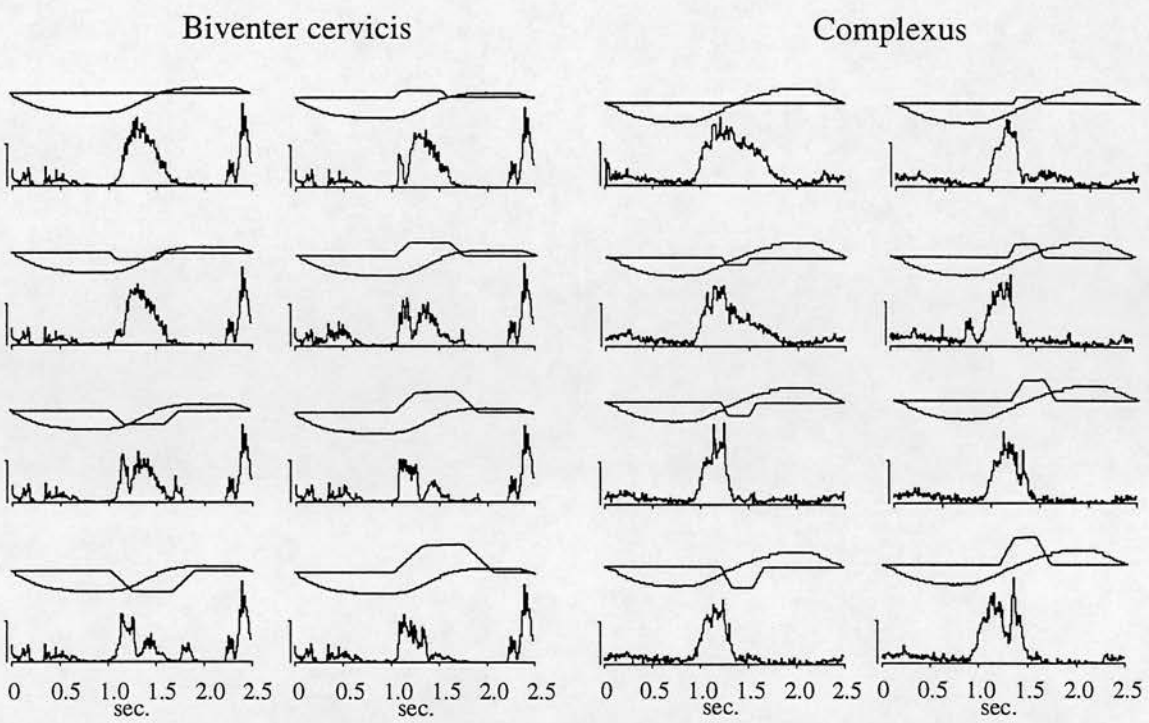


Figure 2.39. Sets of eight interleaved cycle histograms showing the effect of saccadic imposed eye movement (sIEM) of the left eye directed vertically upwards and downwards on the left *biventer cervicis* (left-hand side) and left *complexus* (right-hand side) during sagittal vestibular stimulation. While both muscles are inhibited by sIEM, *biventer cervicis* is apparently inhibited by the S2 portion of the sIEM unlike the effect of sIEM on *complexus* (see text). Scale bars for CHSTs 15.6 μV (*complexus*), 31.5 μV (*biventer cervicis*).

Effects of the artificial VOR

The aVOR with velocity errors initially directed vertically upwards produced reductions in the gain of the vestibular response of *complexus* and *biventer cervicis* in a similar way to that seen during horizontal and frontal vestibular stimulation. Three out of five experiments using the aVOR with velocity errors on *complexus* produced results that had a correlation coefficient (r) of greater than 0.7 when a linear regression was plotted from the data. Three out of three experiments using the aVOR with velocity errors on *biventer cervicis* produced correlation coefficients of greater than 0.7 when the data was plotted and analysed as a linear regression.

The aVOR with phase errors had a much smaller effect during pitch rotation than was seen with horizontal or frontal vestibular stimulation. In the majority of experiments, both on complexus and biventer cervicis no consistent effect was seen, however, a small number of experiments did show an inhibition of the muscle's VCR response below that seen at the compensatory aVOR with phase lags and a slight increase when an aVOR with a phase lead was imposed.

The effect of IEM on contralateral muscles was not investigated during vestibular stimulation in the sagittal plane.

Control experiments

A number of control experiments were performed to ensure that the effects observed with imposed eye movements were due to stimulation of EOM proprioceptors and not stimulation of corneal noci- or mechanoreceptors, retro-orbital mechanoreceptors or cutaneous receptors.

As noted in the methods section, local anaesthetic was routinely applied to the eye before the suction contact lens was placed on the eye and at intervals thereafter. When the lens was placed on the eye in the absence of local anaesthetic, imposed eye movements often caused brief excitations of a muscle's vestibular response that were abolished by the application of local anaesthetic (see Figure 2.40). These phasic excitations were also noticeable as 'shrugs' of the neck muscle during the imposed eye movement. Such reflex activity was never observed during experiments in which the inhibitory effects of IEM described above were observed. Similar excitations and shrugging were, however observed upon pinching the eyelids, or skin around the eye, beak and neck.

The contact lens used to impose movements on the eye was attached to the cornea and sclera by suction. If the suction was removed, allowing the lens to move over the eye and eyelids, no effects on the vestibular response of muscles were observed during imposed movements of the lens (Figure 2.40). The possibility of visual cues producing the observed effects was reduced by making the contact lens opaque. Experiments performed in darkness to prevent visual cues from the uncovered eye affecting the results produced identical results to those seen in the normally lit laboratory. Early control experiments in the pigeon had shown that results on single units in the paralysed pigeon were unaffected by retinal block (Donaldson and Knox, 1990a). This was not repeated in the current series of experiments.

Retrobulbar injection of local anaesthetic to anaesthetise the EOM removed the effects of imposed eye movement on the vestibular response of neck muscles (Figure 2.41). The effects of IEM returned as the anaesthesia wore off. Section of the ophthalmic branch of the trigeminal nerve permanently abolished the effects of IEM on the vestibular responses of neck muscles (Figure 2.42).

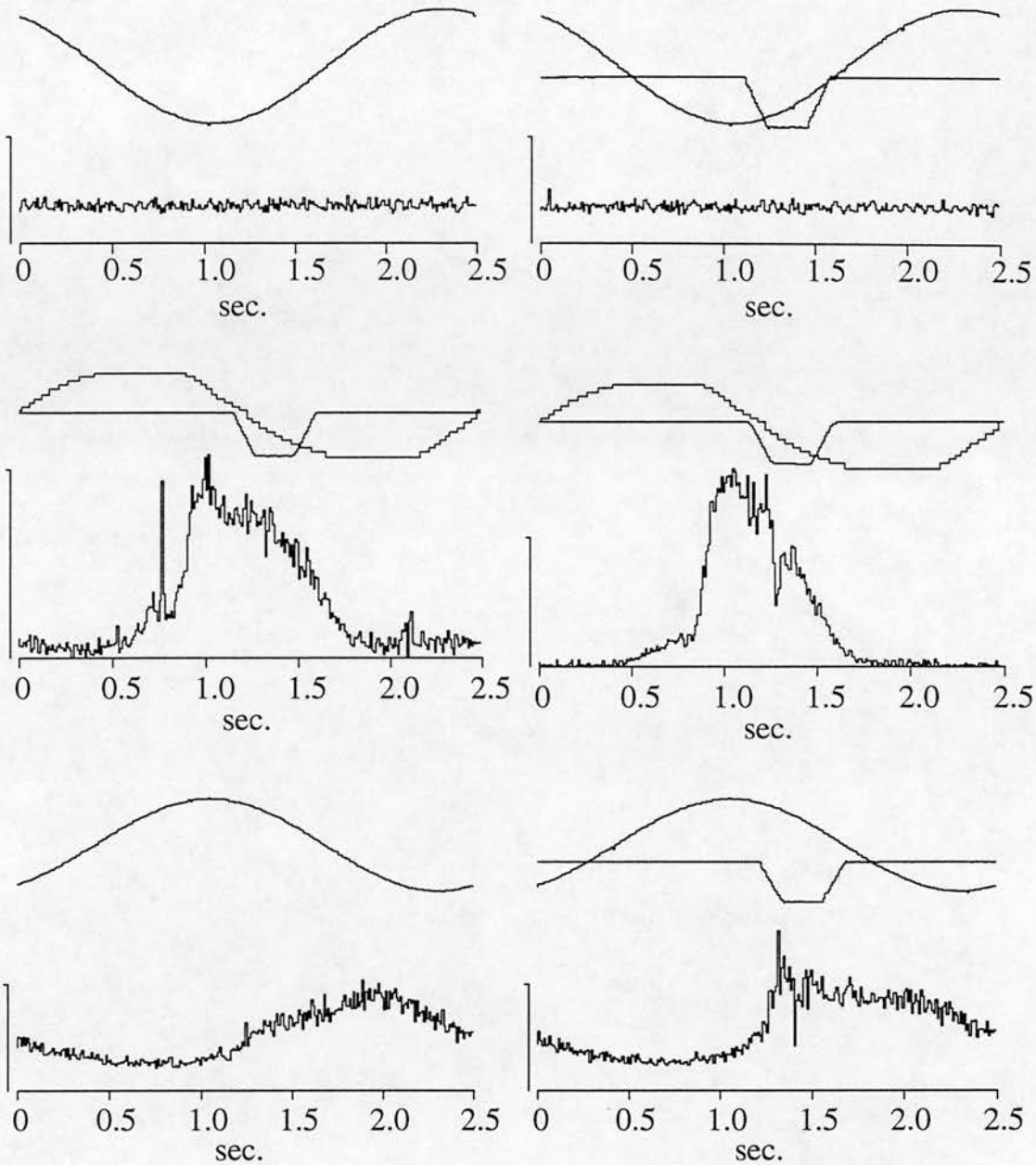


Figure 2.40. Control experiments to eliminate possible sources of error. Top two interleaved cycle histograms (CHSTs) show the lack of an effect of saccadic imposed eye movement (sIEM) of the left eye on the background activity of the left *splenius* during horizontal, sinusoidal vestibular stimulation (VEST) (top right CHST) compared to the background activity during VEST alone (top left CHST). Scale bars for CHSTs 6.4 μV. Middle two CHSTs show the lack of an effect of sIEM of the right eye during frontal VEST on the VCR response of the right *complexus* when there was no suction holding the contact lens to the eye (middle left CHST) compared to the effect seen when suction was applied (middle right CHST). Scale bars for CHSTs 12.4μV. Bottom two CHSTs show the excitation produced by sIEM of the left eye during horizontal VEST on the VCR response of the left *complexus* when there was no local anaesthetic applied to the eye (bottom right CHST) compared to the VCR response during VEST alone. This type of excitation was abolished by application of local anaesthetic. Scale bars for CHSTs 6.3 μV.

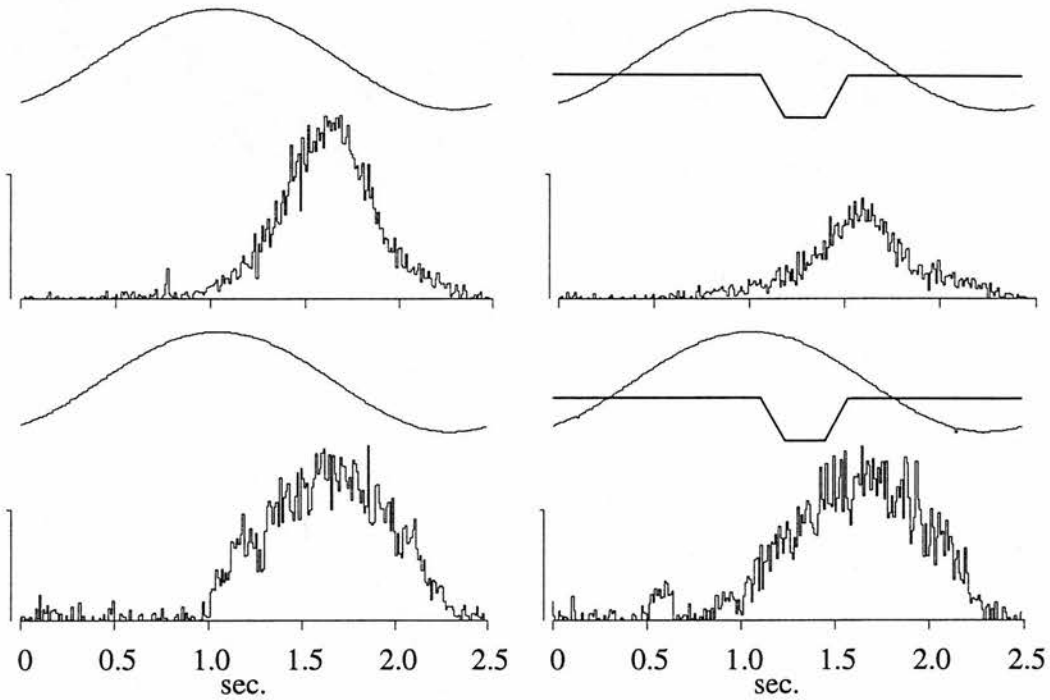


Figure 2.41 Effect of sIEM of the left eye on the VCR response of the left *complexus* before (top) and after (bottom) retrobulbar anaesthesia (1% lignocaine) of the left eye. The inhibitory effect of sIEM returned approximately one hour after the injection. Scale bars for CHSTs 32μV.

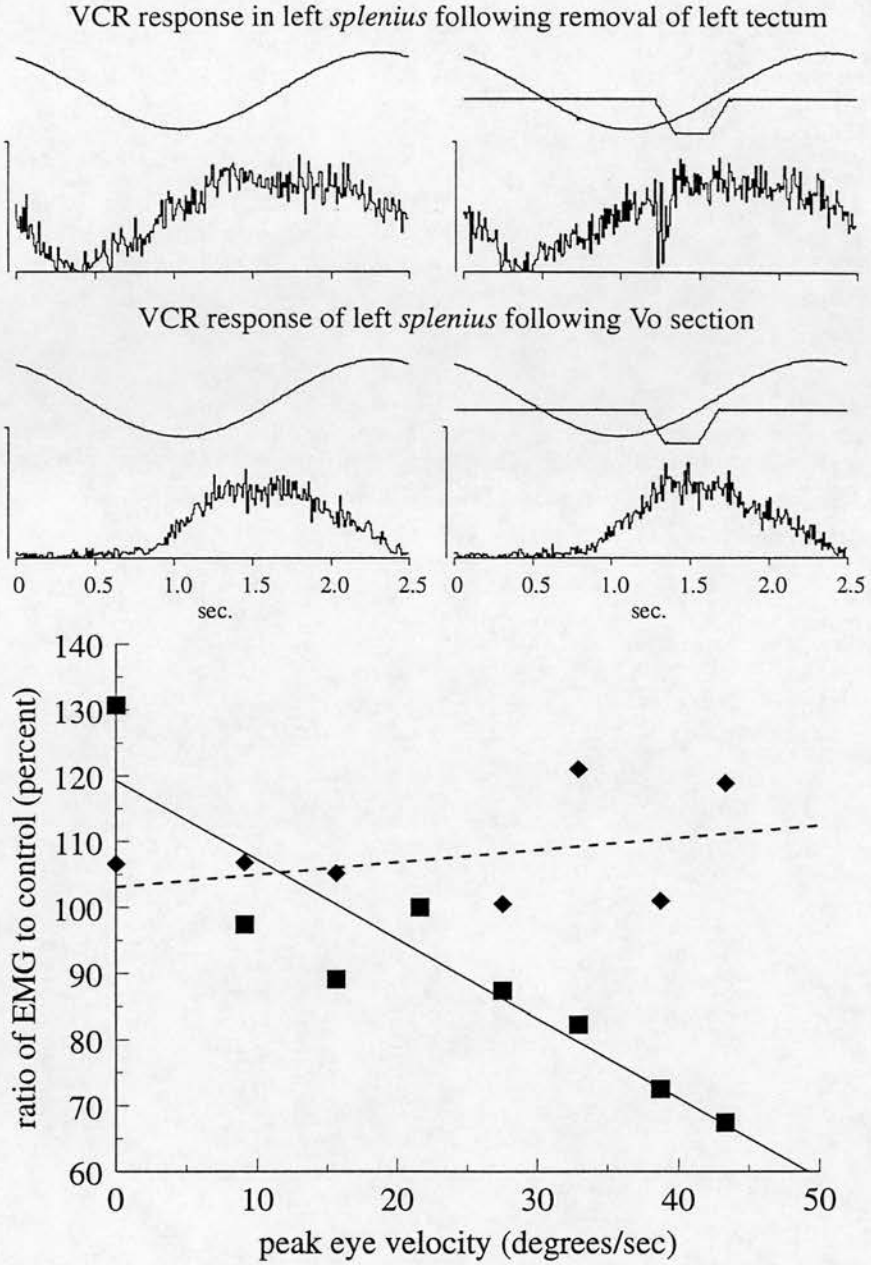


Figure 2.42. Effect of section of the ophthalmic branch of the Trigeminal nerve on the inhibitory effect of saccadic imposed eye movement (sIEM) on the VCR response of the left *splenius* during horizontal, vestibular stimulation (VEST). Top interleaved cycle histograms (CHSTs) show the effect of sIEM (top right-hand CHST) compared to VEST alone (top left-hand CHST) before section of the ophthalmic branch, but after removal of the left optic tectum. Bottom CHSTs show the abolition of any effect of sIEM following section of the ophthalmic branch (bottom right-hand CHST) and the similarity of the VCR response with sIEM to that with VEST alone (bottom left-hand CHST). Scale bars for CHSTs 6.4μV. The graph in the lower half of the figure shows linear regression plots of two experiments imposing the artificial VOR (aVOR) with amplitude/velocity errors before (squares) and after (diamonds) section of the ophthalmic branch of the Trigeminal nerve. The slope of the regression line (solid line) for the experiment performed before the section is -1.21 ($r = 0.92$, $P<0.001$) which is very similar to that obtained for the combined regression line (Figure 2.21) for all experiments in which the aVOR was imposed during horizontal VEST. The slope of the regression line (dotted line) for the experiment following the section is 0.19 ($r = 0.35$, $P>0.05$), the effect of the aVOR on the VCR response has been abolished.

2.4 DISCUSSION

The frequency responses of the neck muscles studied are strikingly different from those found in similar studies in the cat (Shinoda & Yoshida, 1974; Bilotto et al, 1982; Dutia & Hunter, 1985) which showed responses in phase with position at low frequencies and in phase with acceleration at high frequencies. The responses of pigeon neck muscles are very similar to those seen in the extraocular muscles of the pigeon (see Chapter three) and cat, with responses approximately in phase with velocity and showing a small phase lead at low frequencies and a small phase lag at high frequencies. This similarity between neck and extraocular muscle (EOM) responses is not, however, surprising when one considers the pigeon's light head with its low inertia compared to that of the cat. Indeed Outerbridge (1979) and Gioanni (1988) have shown that the pigeon uses its head to stabilize gaze to a considerable extent even at high frequencies, whereas the cat almost exclusively uses its eyes. One may hypothesise that the neural wiring of the pigeon's vestibular gaze system is considerably simpler than that of the cat. Vestibular neurones may well predominantly branch both to the oculomotor and neck motor areas in the brainstem and cervical spinal cord in the pigeon, the vestibulo-oculo-collic neurones described by Berthoz et al (1992) and Peterson et al (1992) in the cat. The development of purely vestibulo-ocular and vestibulo-collic neurones in the cat and other mammals may well simply represent the vestibular apparatus necessary to move a larger, heavier head and far lighter eyes in phase. The fact that the frequency responses of neck and extraocular muscles are similar may be further related to the presence of fast twitch and slow tonic muscle fibres in avian neck muscles (Page, 1969), something that is not observed in the cat's neck musculature, but is seen in the extraocular muscles (EOM) of all species so far examined, including the cat and pigeon.

The slope of the pooled regression for the artificial VOR (aVOR) with amplitude and velocity errors during horizontal vestibular stimulation was very similar to those found for vestibularly modulated single-units in the brainstem and the movement of the globe during horizontal vestibular stimulation. The fact that the aVOR affects the movement of the globe and the activity of dorsal neck muscles in a very similar way provides further evidence that the pigeon neck and eye muscles receive a similar, if not identical, vestibular drive signal.

The effect of imposed eye movement (IEM) on the vestibular activity of dorsal neck muscles studied in the present experiments clearly shows that EOM afferent signals do reach dorsal neck muscles and convey specific information about

the parameters of the IEM. Thus, the vestibular activity of dorsal neck muscles was affected in specific ways related to the direction, amplitude and velocity of the IEM. Such highly specific effects are consistent with an extraretinal signal from EOM proprioceptors of high fidelity, which Sperry (1950) postulated was necessary for the maintenance of visual localisation and, therefore, suggest that EOM afferent signals provide the CNS with accurate information about the movements of the eyes.

The effects of IEM on dorsal neck muscles during vestibular stimulation provide information not only about the signal carried by EOM afferents, but also about the effect that these afferent signals have on particular neck muscles. The absence of an effect of IEM on the spontaneous activity of any of the muscles studied suggests that the modification of electromyographic activity in these muscles may be partly dependent on activity in the EOM, as proposed by Lewis and Zee (1993). Furthermore, the absence of an effect of IEM on *biventer cervicis* during horizontal vestibular stimulation, when no vestibularly-evoked activity was present in the muscle, but a VOR would certainly have been present in the EOM, makes it extremely unlikely that the effects of IEM are a function of activity solely in the EOM. This is in agreement with the results of Donaldson and Knox (1990a, 1991, 1993) in the paralysed pigeon where passive eye movement produced modifications of vestibular activity in brainstem neurones when there would have been no functional VOR due to muscular paralysis. It is, therefore, possible that the EOM afferent signal induced by IEM interacts with the vestibular drive signal to the neck muscles, possibly at the level of the vestibular nuclei, rather than the α-motor neurone. Certainly the results of Donaldson and Knox (1990a) show that an EOM afferent signal induced by passive eye movement does reach and modify single-unit activity within the vestibular nuclei. It would be interesting to know whether non-vestibular neck muscle activity, such as the CCR, is affected by IEM in a similar manner to that seen during vestibular stimulation.

The effect of saccadic IEM (sIEM) on the vestibular response of neck muscles showed 'directional tuning'. Table movement in one direction (for example, to the right) evokes a compensatory VOR activating EOM to move the eyes in the opposite direction (to the left). The sIEMs that produced the greatest inhibition in the VCR response of the neck muscles were directed in the same direction as table movement, and therefore in the opposite direction to the compensatory eye movement which the VOR produces, i.e. the sIEM stretched the EOM in one direction while they were contracting to move the eye in the opposite direction.

Thus, during horizontal oscillation, sIEM towards the beak when the EOM were contracting in an attempt to move the eye towards the tail produced larger inhibitions than IEMs towards the beak (Figures 2.10 & 2.14). Corresponding effects were found in the frontal plane for *complexus* and *rectus capitis ventralis lateralis*. When the table moved upwards the EOM contracted in an attempt to move the eye downwards; sIEM directed vertically upwards stretched the contracting EOMs and moved the eye in the anticompensatory direction (in the direction of table movement) producing considerably larger inhibitions than sIEM directed vertically downwards. However, *splenius* did not show 'tuning' for vertically directed eye movements, with sIEM directed vertically upwards or downwards producing similar inhibitions in the vestibular response. *Biventer cervicis* showed a different pattern of directional tuning for vertically directed sIEM, with sIEM directed in the same direction as table movement producing an increase in the vestibular response above that seen when there was no eye movement (control) in both the ipsilateral and contralateral muscle.

Of course we do not know whether the directional effects are due to this peripheral interaction of stretch and muscle contraction or to central interactions (or both). Previous studies have shown that passive eye movement affects the vestibular modulation of single units in directionally specific ways in the brainstem of paralysed pigeons (Donaldson and Knox, 1990a), so central, 'directional' interactions certainly occur.

An interesting difference between the effects of IEM on *splenius*, *complexus* and *rectus capitis ventralis lateralis* and on *biventer cervicis* is that both ipsilateral and contralateral biventer cervicis muscles increased their VCR responses when eye movements directed vertically upwards were imposed (Figures 2.30 and 2.31). This is in contrast to the other three muscles studied which showed complementary tuning between the ipsilateral and contralateral muscles, e.g. the ipsilateral *complexus* was inhibited to the greatest extent by sIEM directed vertically upwards, whereas the contralateral muscle showed the largest inhibition with sIEM directed vertically downwards.

Furthermore, during VEST in the sagittal plane, the effect of sIEM on the ipsilateral *complexus* and *biventer cervicis* muscles was quite different. *Complexus* showed inhibition to sIEM, the magnitude of which was dependent on the amplitude of the eye movement and which occurred approximately 50 msec after the beginning of the initial, S1, movement, much as was seen in the horizontal and frontal planes. *Biventer cervicis*, on the other hand, while also being inhibited by sIEM, was affected not by the S1 portion of the trapezoidal IEM but by the S2 portion (Figure 2.39).

One must be cautious about reading too much into the effects seen in the sagittal plane because the pigeon would normally make torsional eye movements when moving its head in this plane, the effects of vertical sIEM on the VCR response may well not reflect the 'expected' afferent signals from the EOM. The surprising results of these experiments may simply reflect the 'unusual' stimulus.

This provides further evidence that the effects of sIEM on *biventer cervicis* are significantly different from those seen in the other three muscles studied. One might hypothesise that this muscle responds to different parts of the afferent signal and, possibly, to afferent signals from only certain EOM. *Biventer cervicis* is predominantly a neck extensor in the sagittal plane, and thus may respond to signals from the two vertical recti and the two oblique EOM in preference to those from the horizontal recti; however, this remains conjecture and something worthy of considerable further study.

The artificial VOR (aVOR) has proved an extremely useful method of producing a more physiological method of imposed eye movement than sIEM. One of the most notable effects of the aVOR is the lack of an effect on the phase of the vestibular response (Figure 2.22), whatever the parameters of the aVOR. The effect of the aVOR was solely on the gain of the response. With the eye being moved throughout the vestibular stimulus cycle, the aVOR affects the whole of the response and not a part of it as was seen for sIEM.

The two manipulations of the aVOR which were used were changes in the amplitude/velocity and in the phase. Changing the amplitude and velocity of the aVOR produced systematic changes in the vestibular response (see Figures 2.20, 2.21, 2.33, 2.34 and 2.35). This result suggests that the EOM afferent signal may have a corrective effect on the VCR response of neck muscles. Movements of the eye at velocities below that of the compensatory VOR increased the gain of the VCR. If the head were free to move, an increased VCR gain would limit head movement to correspond to the reduced eye movement, and this would tend to produce a stable gaze direction. Conversely, when the imposed eye velocity exceeded that needed for compensation, the gain of the VCR response was reduced. This, again, would tend to stabilize the direction of gaze if the head were free to move; a decreased VCR gain producing increased head movement allowing the head to 'catch up' with the eye. The effect of the aVOR with amplitude and velocity errors on the contralateral *biventer cervicis* during frontal VEST was unlike that seen for the other contralateral muscles during frontal or horizontal VEST, with increasing amplitudes and velocities increasing the activity within the muscle above that seen when the eye was held still.

This apparent corrective effect of the aVOR with amplitude and velocity errors is an attractive functional hypothesis, but it may not explain the data from other experiments. The reversal of the expected effect of the aVOR on the contralateral *biventer cervicis* appears to contradict any simple functional theory, such as the corrective hypothesis proposed above. This result suggests instead that the aVOR is, in fact, merely another example of the effects of amplitude and velocity seen with sIEM. The VCR response of the contralateral biventer cervicis was increased by sIEM directed vertically upwards during the portion of the vestibular stimulus cycle when the muscle was active. The aVOR with amplitude and velocity errors also moved the eye upwards during this portion of the vestibular stimulus cycle. The other muscles studied were inhibited by sIEM directed vertically upwards and so the effect of the aVOR was, not surprisingly, an increase in the magnitude of the inhibition with increasing amplitude and velocity.

The effect of altering the phase rather than the amplitude and velocity of the imposed aVOR cannot be explained simply in terms of a corrective effect. A simple corrective drive to neck muscles produced by EOM afferent signals would increase the VCR activity of neck muscles when an aVOR with a phase lag was imposed, in effect to increase the stability of the head and allow the eye to 'catch up' with the head, and, conversely, decrease VCR activity during an aVOR with a phase lead allowing the head to 'catch up' with the eye. This effect on VCR activity, however, was not observed when the aVOR with phase errors was imposed. In fact, the aVOR with phase leads produced an increase in the VCR activity above that seen when imposing the compensatory aVOR and, with phase lags, a decrease. This would, initially, appear to be the reverse of what a functional, corrective hypothesis would suggest. The aVOR with phase errors elicits a complicated error signal. While the peak amplitude of the imposed eye movement is constant (that of the compensatory aVOR) the position and direction of imposed eye movement when phase errors are introduced varies considerably from the movement of the globe the EOMs are trying to produce. Thus with phase leads the eye is moved ahead of the motorneurone drive, 'unloading' the muscles that are contracting, and with phase lags the eye is moved behind the motorneurone drive, the EOMs thus contracting against a lagging eye, in effect being stretched. As seen with IEM at saccadic velocities, the combination of EOM contraction and stretch produced the greatest inhibition in the VCR response. The aVOR with phase errors also produces changes in the direction that the eye is being moved. As the vestibular turntable changes direction, so the vestibular drive to the EOM changes to move the eye in the opposite direction. The compensatory aVOR mimics this in the sinusoidal movement it imposes on the eye.

The aVOR with phase errors changes the point in time within the vestibular stimulus cycle that this 'changeover' in the direction of imposed eye movement occurs. With an aVOR with a phase lag the change in direction of eye movement will be delayed so that, for a portion of the vestibular stimulus cycle when the vestibular drive to neck muscles is at its highest, the eye will have movement imposed in the opposite direction to that 'expected'. Imposed movements of the eye in the opposite direction to that of a compensatory VOR were shown to produce the greatest inhibition for IEM at saccadic velocities; the aVOR with phase errors is, in part, a corollary of this for slow, sinusoidal eye movements.

A further finding of the experiments described above is the different effects that IEM had on the four neck muscles studied and the relation that this appears to have to their planes of action. The most striking example of this is *biventer cervicis* which did not show any vestibularly-evoked activity during vestibular stimulation (VEST) in the horizontal plane and there was no effect of IEM on the spontaneous activity in this muscle during horizontal VEST. However, in the frontal and sagittal planes there was considerable vestibularly-evoked activity which was modified to a considerable degree by IEM.

The increase in the VCR response of the ipsilateral *biventer cervicis* in response to imposed eye movements directed vertically upwards is notably similar to the finding of Roucoux et al (1989) that in the head-fixed cat the ipsilateral *biventer cervicis* increased its level of EMG activity when the eye was directed vertically upwards. Similarly, Roucoux et al (1989) showed in the cat that the ipsilateral *splenius* and *complexus* muscles were excited by eye movements towards the ipsilateral side (in the horizontal plane) and vertically downwards. IEM directed towards the ipsilateral side produced the smallest inhibitions in the VCR responses of *splenius* and *complexus*. *Complexus* was also inhibited to a far lesser extent by IEM directed vertically downwards than IEM directed vertically upwards. The excitation seen with IEM directed vertically upwards in *biventer cervicis* was reduced by increasing amplitudes of IEM. It is possible that an excitatory drive to *splenius* and *complexus* due to the direction of IEM was masked by a larger inhibitory signal due to the amplitude of IEM, making the results seen with IEM in the decerebrate, head-fixed pigeon remarkably similar to those reported in the alert head-fixed cat.

Rather than showing the direction of greatest excitation of Roucoux et al, the effects of IEM on *complexus*, *splenius*, *rectus capitis ventralis lateralis* and *biventer cervicis* can more accurately describe the direction, or directions, for which the maximum inhibitory signal is produced by EOM afferents. The four muscles studied

each showed different and quite specific responses to the various directions of IEM. The ipsilateral *complexus* showed the greatest inhibition with IEM directed vertically upwards during frontal VEST and with IEM directed both towards the beak and vertically downwards during horizontal VEST. The contralateral *complexus* showed 'complementary tuning', being inhibited to the greatest extent by IEM directed vertically downwards in the frontal plane and directed both towards the tail and vertically downwards during horizontal VEST. The ipsilateral *splenius* showed 'finer' tuning in the horizontal plane, being inhibited to the greatest extent by IEM directed towards the beak, and little tuning in the frontal plane, being strongly but almost equally inhibited by IEM directed either vertically upwards or downwards in the frontal plane. Similarly, the contralateral *splenius* showed very discrete tuning in the horizontal plane, towards the tail, during horizontal VEST, but little preference for direction during frontal VEST. The ipsilateral *rectus capitis ventralis lateralis* (*r.c.v.l.*) was most inhibited by IEM directed vertically downwards during horizontal VEST and directed vertically upwards during frontal VEST. The ipsilateral *biventer cervicis* was inhibited to the greatest extent by IEM directed vertically downwards during frontal VEST and showed no vestibular response during horizontal VEST and the contralateral *biventer cervicis* was also inhibited to the greatest extent by IEM directed vertically downwards during frontal VEST.

It is tempting to compare these preferential directions to the actions of the four muscles studied. While *biventer cervicis* showed no VCR response in the horizontal plane and therefore obviously does not participate in head movements or stabilization in this plane, *complexus*, *splenius* and *r.c.v.l.* all have VCR responses. The differences in the effect of IEM on these three muscles is consistent with their probable actions. *Splenius* probably produces a greater force for active head movements in the horizontal plane (and a smaller one in the frontal plane) due to its more horizontal plane of action, with *complexus* and *r.c.v.l.*, both of whose plane of action lies far more in the vertical plane stabilizing the head rather than creating much active force for head movement. This too is consistent with the effect of vertical IEM on these two muscles during horizontal VEST. Conversely *complexus* and *r.c.v.l.* are well suited to movements in the frontal plane, as is *biventer cervicis*, whose longitudinal midline position obviously prevents any participation in horizontal head movements.

Statistical analysis of the results of imposed eye movement on the activity of dorsal neck muscles

All of the experiments discussed in the present chapter are designed round a system of interleaving stimulus presentation and data collection (see section 2.2.4) which allows a comparison of the effects of several (usually eight) stimulus conditions as though they had all been delivered simultaneously. Its great strength is that it greatly reduces the effects of variability of the excitability of central processes over time. All the plots and histograms are derived from single interleaved sets in which one stimulus condition is vestibular stimulation alone and the others are vestibular stimulation plus imposed eye movement (IEM) at various velocities, amplitudes and so on. The figures included in this chapter are examples of the effects of different types of IEM on the vestibular response of various neck muscles and there were many such examples, as described in the text. It initially seemed attractive to consider constructing graphs of the average response to a particular type of IEM and producing error bars as for the graphs of the frequency responses of the muscles studied (Figures 2.6 & 2.7).

However, to average the results from different sets would immediately introduce uncertainties because the sets were collected at different times. Notwithstanding this, if one were to 'average' the results of a number of sets it would first be necessary to normalise the data of each set with respect to its own control, the electromyographic activity during vestibular stimulation alone, as was done for graphs constructed from single sets. This creates a group of ratios derived from several different sets. Each group of ratios for a particular type of IEM could then be expressed as an average, but the variances of ratios are not simply additive; var A + B = var A + var B but var A / B (the variance of a ratio) is not equal to the sum, difference or ratio of the individual variances. So one cannot derive the error bars unless another whole set of assumptions about the distribution of the variances of the ratios is made and obviously error bars cannot be derived from a single set.

Error bars, and the like, add 'confidence' to data. The results of the present experiments showed considerable consistency both within individual experiments and between preparations. While the magnitude of the effects of IEM varied between experiments and preparations, there were no experiments in which, for example, the 'directional tuning' of a particular muscle was reversed. There were preparations that showed little effect of IEM on neck muscle activity, but these also showed unusual frequency responses to vestibular stimulation alone, suggestive of damage to the brainstem during the initial decerebration.

Testing the statistical significance of pairs of observations is possible using the method of Dörrscheidt, and the figure legends do contain the results of this test where different directions of IEM were used in a single set. Almost invariably Dörrscheidt's test shows statistical significance when two directions of IEM are compared, usually at the 0.1% level of significance. It must be remembered that statistical significance does not necessarily relate to biological significance. In the present experiments the effects of IEM are very obvious to the naked eye and this is merely reflected in the very marked levels of statistical significance.

Choice of stimulus

Many studies on the effects of EOM proprioceptors have used stretch of a single EOM. IEM has many advantages over this type of stimulus. Apart from technical difficulties concerning keeping the isolated muscle preparation working for long periods of time and ensuring that stretch of one muscle is not also stimulating other EOM via their tendinous attachments at the annulus of Zinn, stretch of a single EOM is quite unlike the pattern of stretch, contraction and relaxation in all six EOM in most, if not all, natural eye movement. IEM was, therefore, used as the stimulus to activate EOM receptors because it produces movements of the globe that mimic natural eye movements, although the exact mechanics of the EOM, in which some are contracting to produce eye movements, are not mirrored by IEM. IEM also allowed movements of the eye in different directions, presumably producing quite different afferent signals, to be studied, effectively simultaneously, using the interleaved cycle histogram technique. Slippage of the suction contact lens on the globe is highly unlikely, because very similar results were obtained when experiments were repeated, even after a considerable amount of time, and such slippage would have been very obvious as a decrease in the level of suction as measured by the manometer used to control the level of suction.

Source of the afferent signal induced by imposed eye movement (control experiments)

The various control experiments performed confirm that the effective signal produced by imposed eye movement (IEM) originates in the extraocular muscles (EOM) and, almost certainly, from proprioceptors within these muscles.

A visual source was eliminated by repeating a number of experiments in complete darkness, which had no effect on the experimental results. Donaldson and Knox have previously shown that pharmacological block of the retina has no effect

on the responses of single units in the brainstem to passive eye movement (Donaldson and Knox, 1990a). The possibility of an auditory source was eliminated by ensuring that the vestibular tables and eye-movers were silent and that sudden noise (e.g. hand-claps) produced no change in the VCR response. Cutaneous signals were shown to have no effect on the VCR response by the placing the lens on the eye without suction being applied, so that the lens was running over the eye and under the eyelids (Figure 2.40). Local anaesthetic regularly placed onto the cornea removed any responses from corneal afferents, which were very obvious when present as 'sharp' excitations in the VCR response during the phasic portions of sIEM, unlike the predominantly inhibitory effects otherwise produced by IEM (Figure 2.40).

One of the most compelling pieces of evidence that the effects of IEM are due to stimulation of EOM proprioceptors is the very nature of these effects on neck muscle vestibular responses. IEM produces very consistent effects on neck muscle VCR responses, with different directions of IEM producing very different effects. Increasing amplitudes or velocities of IEM produced systematic increases in the inhibition of the VCR response, which is consistent with the afferent signal that a receptor responding to changes in length or to stretch would produce in response to such increasing stimuli. If these were the only responses to have been found, it could, however, be argued that these effects were due to other receptors around the orbit, such as mechanoreceptors or nociceptors, or that the effects were due to the force being exerted on the suction cup at any one moment, possibly changing the state of arousal of the brainstem by what in a conscious animal would simply be pain, greatest when the eye is being forced in a different direction to where its muscles are pulling it. These objections are overcome by the effects of IEM on the contralateral muscle, where IEM in the opposite direction to that which produced the greatest inhibition in the VCR response of the ipsilateral muscle produced the largest inhibition in the VCR response of the contralateral muscle (e.g. Figure 2.16). It seems highly unlikely that extra-orbital receptors would consistently produce inhibitions in an ipsilateral muscle for one direction of IEM and not in a contralateral muscle, unless these receptors were specialised to provide accurate information on the position or movement of the globe. It is improbable that there are such extra-orbital receptors, certainly none have been found in many detailed studies of the orbital cavity, whereas stretch receptors have been identified in the EOM of many animals that are very good candidates for the source of the specific eye-movement-related afferent signals reflected in the effects of IEM on VCR responses in neck muscles. Further support for the effective signal originating in EOM proprioceptors

comes from the quite different effects produced by IEM on the VCR response of different neck muscles in the same preparation (e.g. Figures 2.27 & 2.30). That a particular direction of IEM could almost abolish the VCR response of one muscle and yet excite another, as was seen with IEM directed vertically upwards on *complexus* and *biventer cervicis* during roll tilt, argues very strongly against the effective signal produced by IEM being 'painful arousal' or from mechano- or nociceptors.

The removal of any effect of IEM on a muscle's VCR response by section of the ophthalmic branch of the Trigeminal nerve is a further strong piece of evidence that the effective signal produced by IEM originates from the EOM. Whilst the ophthalmic branch is also known to contain afferent fibres from cutaneous receptors and nociceptors surrounding the orbit and beak, signals from these receptors have been shown not to affect the VCR response. It is known that EOM proprioceptive afferents pass from the mixed intraorbital oculomotor nerves to the ophthalmic branch of the Trigeminal nerve before or at the cavernous sinus in many species, including the pigeon (see Chapter 4). Given the highly specific nature of the effects of IEM on neck muscles, highly suggestive of proprioceptive signals as discussed above, the total disappearance of the effects of IEM following ophthalmic branch section strongly suggests that receptors within the EOM are responsible for the effects of IEM on neck muscle vestibular responses and that afferent fibres from these receptors pass through the ophthalmic branch of the Trigeminal nerve on their way to the brainstem (see Chapter 4).

Conclusion

The results of this series of experiments have shown that the four neck muscles studied each respond to afferent signals from the EOM induced by IEM in discrete and specific ways which can be correlated to their different actions in the control of head movement. This is important because it suggests EOM afferent responses provide a functionally significant signal to dorsal neck muscles and not just a relatively non-specific signal that indicates whether the eye has moved or not.

CHAPTER 3. EFFECT OF IMPOSED EYE MOVEMENT ON THE EXTRAOCULAR MUSCLES AND MOVEMENTS OF THE GLOBE DURING THE VESTIBULOOCULAR REFLEX

3.1 INTRODUCTION

Donaldson and Knox (1990a, 1990b, 1991, 1993) have published a series of elegant papers investigating the effects of passive eye movement on vestibularly-modulated single units in brainstem nuclei involved in oculomotor control. They have recently extended these observations by investigating the effects of imposed eye movement on individual extraocular muscles (EOM) and movements of the globe as a whole during vestibular stimulation (Knox and Donaldson, 1991, 1993). Their results in the decerebrate pigeon strongly suggest that EOM afferent signals play a part in the control of eye movements and that this control may be from moment-to-moment.

The effect of deafferenting the EOM by section of the ophthalmic branch of the trigeminal nerve (Vo) is known to produce quite dramatic effects on eye movement. Fiorentini and Maffei (1977) showed that the cat eye became unstable following unilateral section of Vo and that the animal subsequently showed defects in orienting behaviour. Hein and Diamond (1983) showed deficits in visually guided behaviour in deafferented, dark-reared kittens, and Buisseret (1979), following a similar procedure, showed a reduction in the number of binocularly activated cells in the visual cortex (see Chapter 1). Kashii et al (1989) performed unilateral Vo section in the rabbit and found eye oscillations of up to 12° on the operated side when the animal was placed in a dark room as well as a preponderance of anticompensatory eye movements during vestibular rotation. Following a similar procedure Kimura et al (1991) found a large decrease in the VOR gain of the deafferented eye in the rabbit, deficits in the gain of optokinetic nystagmus (OKN) and a reduction in the velocity of quick eye movements of OKN.

The finding that Vo section removed the effects of imposed eye movement (IEM) on the vestibular activity of dorsal neck muscles in the decerebrate pigeon (Chapter 2) suggested that it would be interesting to investigate whether Vo section also abolished the effects of IEM on the vestibular activity of the EOM and what effect this section had on the VOR.

3.2 METHODS

The methods of stimulation, recording and analysis were generally similar to those described in Chapter 2. Briefly, pigeons were decerebrated under ether anaesthesia and were mounted in a headholder centred on the axis of rotation of a horizontal vestibular turntable and arranged so that the horizontal canals lay approximately in the earth-horizontal plane (Erichsen et al, 1989).

3.2.1 Stimulation

Natural vestibular stimulation in the horizontal plane and activation of extraocular muscle (EOM) stretch receptors of the left eye by imposed eye movement were achieved as described in Chapter 2. The gain of the pigeon vestibuloocular reflex (VOR) is approximately constant at -0.9 when tested with sinusoidal oscillation at frequencies between 0.2 and 2.0 Hz (Gioanni, 1988; Anastasio and Correia, 1988) and is independent of stimulus magnitude between 6 and 20°/s. Stimulus magnitudes above 20°/s produce a decrease in the gain of the VOR (Gioanni, 1988; Anastasio and Correia, 1988); the stimulus magnitude in the following experiments never exceeded 20°/s. There is a small phase lead of the eye relative to the head at 0.2 Hz which decreases to approximately 0° at 1.0 Hz.

3.2.2 Recording of EMG

Multiunit electromyograms (EMG) were recorded differentially between pairs of electrodes (stainless steel insect pins partly pushed into fine insulated wire) which were inserted into the *lateral rectus* muscles. The pigeon globe is flattened compared to the spherical globe of mammals, so access to the EOMs which insert behind the outer lip of the pigeon globe was gained by making small incisions in the skin beside the eye, cutting the conjunctiva at the edge of the globe and guiding the EMG electrodes behind the globe. This blind placement of electrodes necessitated dissection of the orbit at the end of an experiment to ensure the electrodes were recording from the *lateral rectus*; this, however, proved to be the case in all the experiments described hereafter. Amplification and collection of the EMG signal were as described in Chapter 2.

3.2.3 Recording of EOG

EOG electrodes were made from Teflon-coated silver wire (AG-3T, Clark Electromedical Instruments, Reading). A small length of Teflon was burnt off both ends of the wire and the silver was melted into a small sphere at one end. Small incisions were made in the skin at the anterior and posterior edges of the left and right eyes, and the overlying connective tissue was removed from the bone. Small holes were made in the bone in approximately the horizontal plane, and the ends of the electrodes were lodged firmly into these. At the end of the experiment the position of the electrodes was checked to ensure that they had remained in place; this was always the case. Because of the construction of the pigeon orbit it was not possible to place the electrodes in exactly the horizontal plane. The anterior electrode was usually slightly below the horizontal and the posterior slightly above the horizontal. It was therefore possible to resolve most, but not all, of the horizontal eye movement.

The signals were amplified between 1000- and 10000-fold with an amplifier band pass (- 3 dB) of 0.3 Hz to 0.3 kHz and fed to the CED 1401 programmable interface controlled from an IBM-AT compatible microcomputer as described in Chapter 2. The collection and analysis of EOG data was the same as that described for collection of EMG data except that the incoming, amplified EOG signal was not rectified.

EOG and table position signals were also digitally recorded on video tape and plotted on a chart recorder to examine cycle-to-cycle variability and check for the presence of fast phases in the signal. The EOG signal was calibrated by placing the suction contact lens on the left and right eye in turn and imposing movements of known amplitude. The signals recorded for a number of amplitudes were plotted as a linear regression, so the amplitude of eye movement in a given experiment could then be calculated from the EOG signal recorded.

3.2.4 Section of the ophthalmic branch of the trigeminal nerve

The method was exactly the same as that described in Chapter 2. Briefly, the left tectum was removed by aspiration and the trigeminal ganglion and ophthalmic branch uncovered by removing the overlying dura. Following removal of the tectum, experiments were repeated to ensure that there was little or no change in the VOR. The ophthalmic branch was sectioned with a small scalpel. The section was confirmed at the end of the experiment by careful dissection of the skull.

3.3 RESULTS

Response of the lateral rectus to natural vestibular stimulation

The frequency response of the vestibuloocular reflex (VOR) in the *lateral rectus* muscle during horizontal vestibular stimulation was studied over a decade of frequencies from 0.2 Hertz to 2.0 Hertz and is shown in Figure 3.1. The gain of the VOR can be seen to increase with increasing frequency, whilst the response is approximately in phase with head velocity.

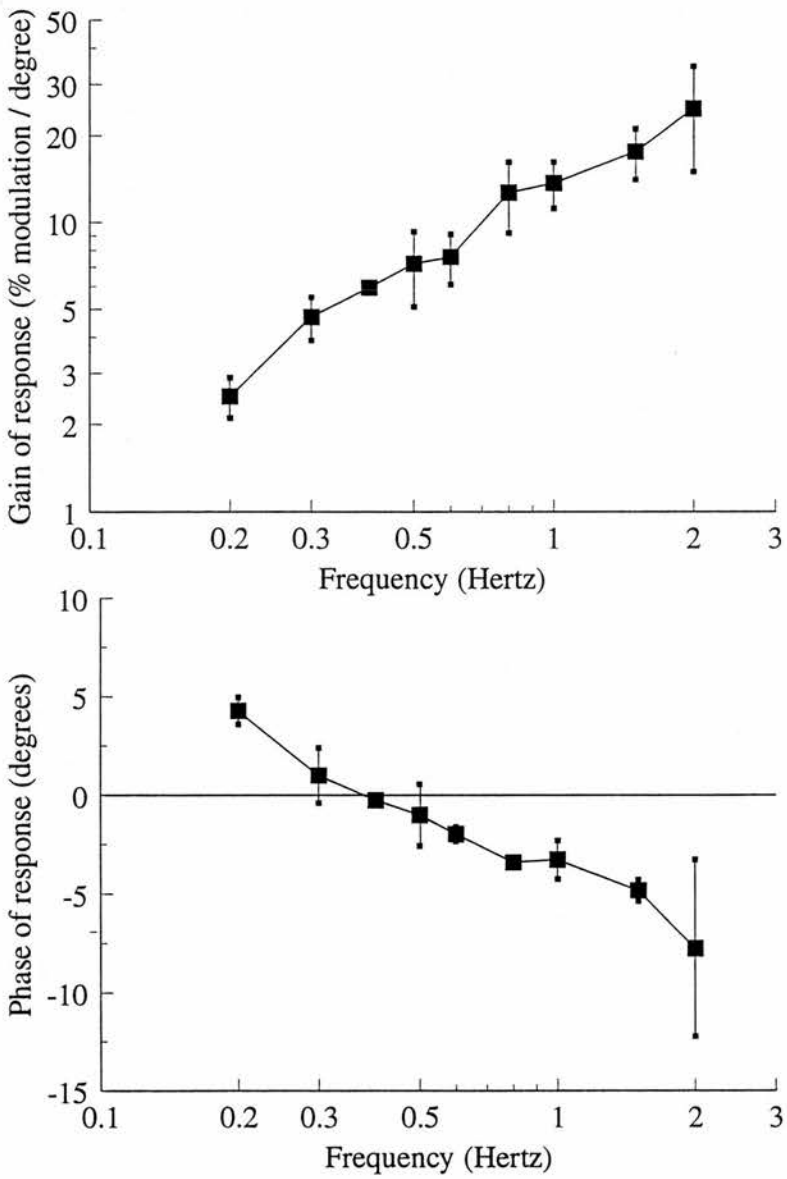


Figure 3.1 Plots of the gain and phase of the VOR-response in *lateral rectus* to sinusoidal, horizontal, vestibular stimulation over a decade of frequency at stimulus magnitudes not exceeding 30°/s (7°/s - 29°/s). Points are mean ± s.e.m. (n=5); scales are log/log for gain, log/linear for phase.

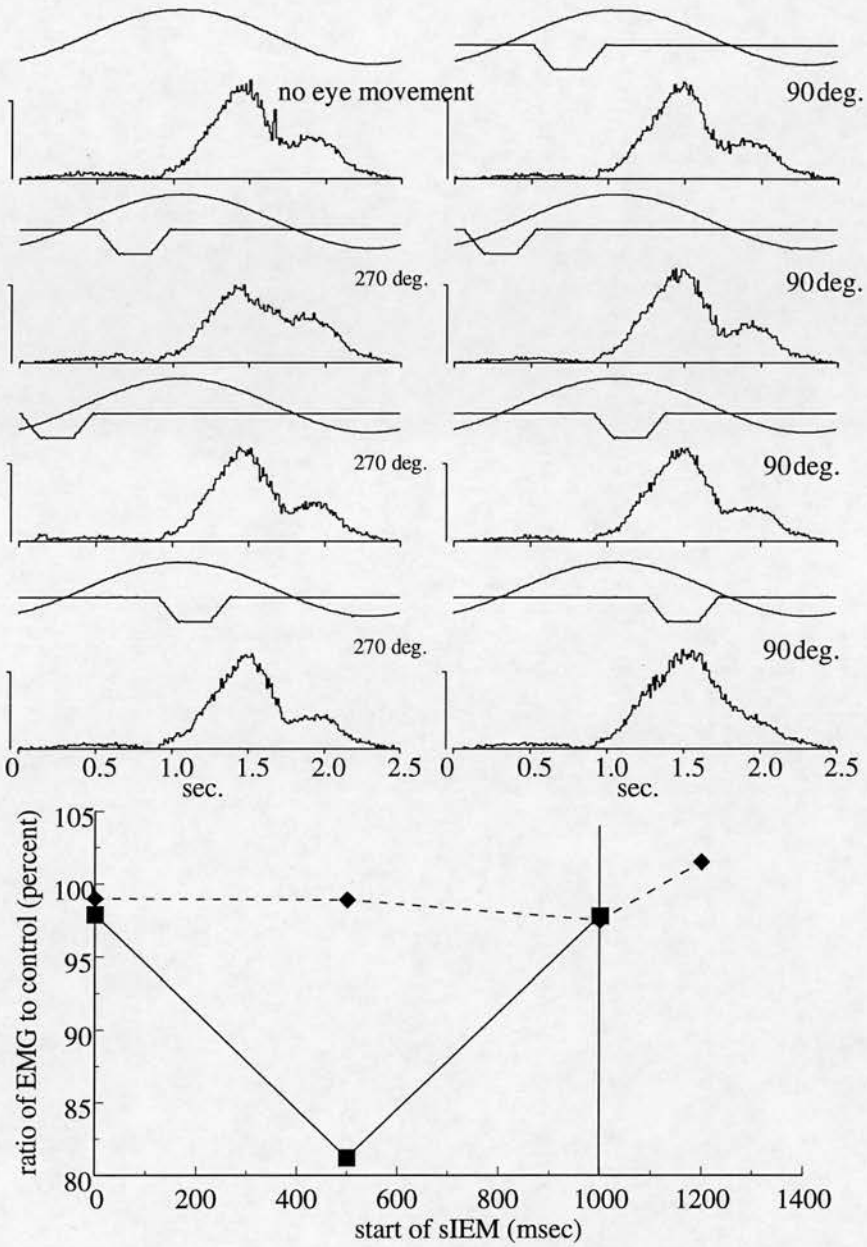


Figure 3.2. Set of eight cycle histograms (CHSTs) showing the effect of different directions of saccadic imposed eye movement (sIEM) of the left eye on the electromyographic (EMG) activity in the right *lateral rectus* during horizontal; vestibular stimulation (VEST ±8° at 0.4 Hz). Each CHST represents exactly one cycle of vestibular oscillation. Upward deflection of vestibular table position trace (sinusoid) represents movement to the right. Eye position trace (solid line) shows the time course of sIEM. The top left CHST shows the response to VEST alone (eye held still). The remaining seven CHSTs show the response to VEST and added sIEM imposed in two directions (towards the right, 270° or towards the left, 90°) at different latencies. Scale bars for CHSTs, 16 μV. The graph in the lower half of the figure is derived from the eight CHSTs and shows the ratio of the modulation of the averaged EMG activity during combined VEST and sIEM, to the modulation of the EMG activity during VEST alone (control), plotted against the start of sIEM (squares represent sIEM towards the right, 270°; diamonds, sIEM towards the left, 90°). The magnitude of the reduction in EMG activity is dependent on the direction of the eye movement, as well as on the time at which it is imposed, with sIEM towards the right (270°) occurring 500 msec after the beginning of the vestibular stimulus cycle having the largest effect. Vertical solid line represents the start of activity in the right *lateral rectus*.

Effects of imposed eye movement on the electromyogram of the lateral rectus during horizontal vestibular stimulation

Imposing movements on the left eye at saccadic velocities (sIEM) inhibited the vestibular activity of the right *lateral rectus*. The magnitude of this inhibition was strongly dependent on the time during the vestibular stimulus cycle at which the eye movement was imposed and on the direction of this sIEM (Figures 3.2 and 3.3).

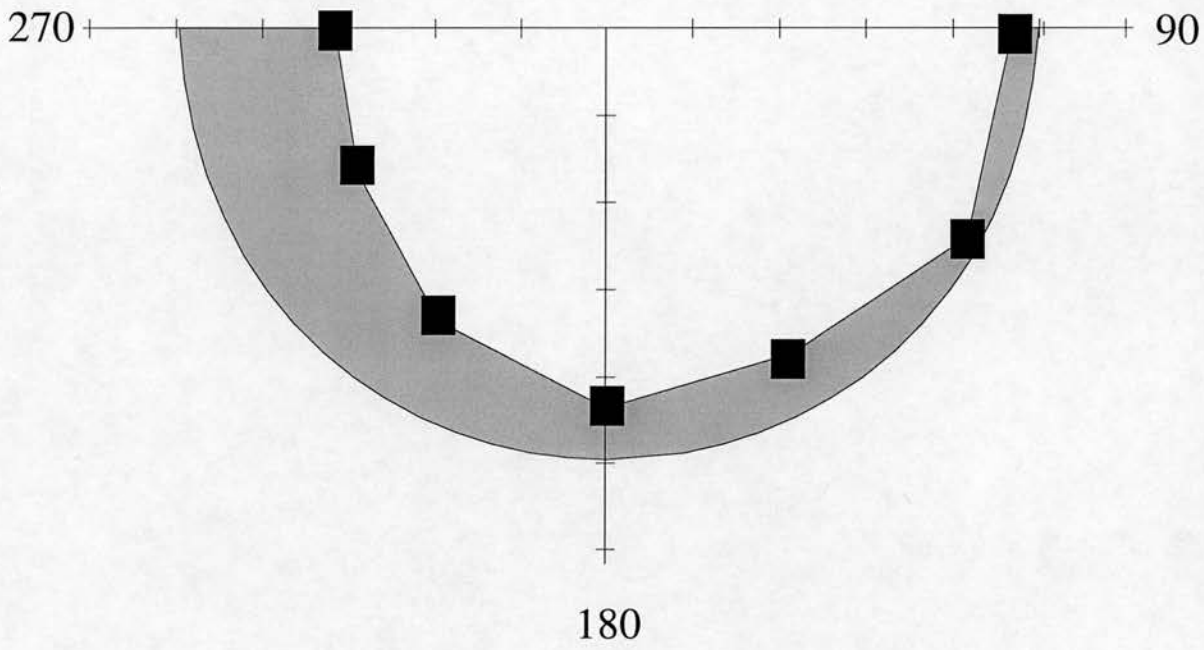


Figure 3.3. Plot derived from a set of eight interleaved cycle histograms (CHSTs) investigating the effect of different directions of saccadic imposed eye movement (sIEM), which was constructed from the modulation of the rectified electromyographic (EMG) activity integrated over the same time window, bins 151-186 (350 ms), for each CHST. 0°/360° corresponds to sIEM initially directed vertically upwards; 60°, upwards and towards the left (tail); 90°, towards the left (tail); 120°, downwards and towards the left; 180°, vertically downwards; 240° downwards and towards the right (beak); 270°, towards the right (beak) and 300° upwards and towards the right. The response to a particular direction of sIEM is plotted as a vector in which the distance of a point from the centre of the plot represents the magnitude of the response, and the angle of the vector shows the direction of sIEM. The circle represents the response to vestibular stimulation in the horizontal plane alone (control). The shaded area shows the inhibition produced by sIEM relative to the control response. The plot shows the response to sIEM imposed approximately one second before the beginning of the VOR response in the right lateral rectus in various directions from towards the left (tail, 90°) to towards the right (270°, beak). Scale divisions for polar plot 1.0 μV.

Imposing an artificial VOR (aVOR) with velocity and amplitude errors produced systematic modulations of the right *lateral rectus*. Imposing an aVOR with an amplitude and velocity below that required to compensate for the vestibular stimulation (the compensatory aVOR) increased the VOR response of the right *lateral rectus* above that seen at the compensatory aVOR, whereas imposing an aVOR with an amplitude and velocity above that of the compensatory aVOR reduced the VOR response below that seen at the compensatory aVOR (Figure 3.4).

The results of five experiments when plotted as linear regressions all had statistically significant correlation coefficients ($r > 0.7$). Analysis of covariance of the regression data for pairs of experiments with the widest spread of parameters and/or largest difference in regression slopes suggested that the regression lines formed one group homogeneous in slope. The mean slope for the combined regression line was -1.05 ($r = 0.807$, $n = 40$) which was statistically significant ($P < 0.01$).

Effect of imposed eye movement on the Vestibuloocular reflex

As was noted by Knox and Donaldson (1993), the VOR gain was very variable in the decerebrate pigeon, varying from -0.3 to -1.2. The VOR gain measured from cycle histograms averaged over 24 sweeps was considerably more stable in an individual bird over long periods of time. The effect of IEM at saccadic velocities on the VOR was not investigated.

Imposing the artificial VOR (aVOR) with velocity and amplitude errors produced similar effects to those seen in both dorsal neck muscles and the *lateral rectus* muscle, increasing peak velocities and amplitudes produced increasing reductions in the VOR (Figure 3.5). The results of individual experiments were plotted as linear regressions, and analysis of covariance of regression data from pairs of experiments with the widest spread of parameters and/or largest differences in slope suggested that the regression lines formed one group homogeneous in slope. The combined regression line gained from pooling the results of five experiments had a slope of -0.97 and the correlation coefficient of this regression line was significant ($r = 0.792$, $n = 40$).

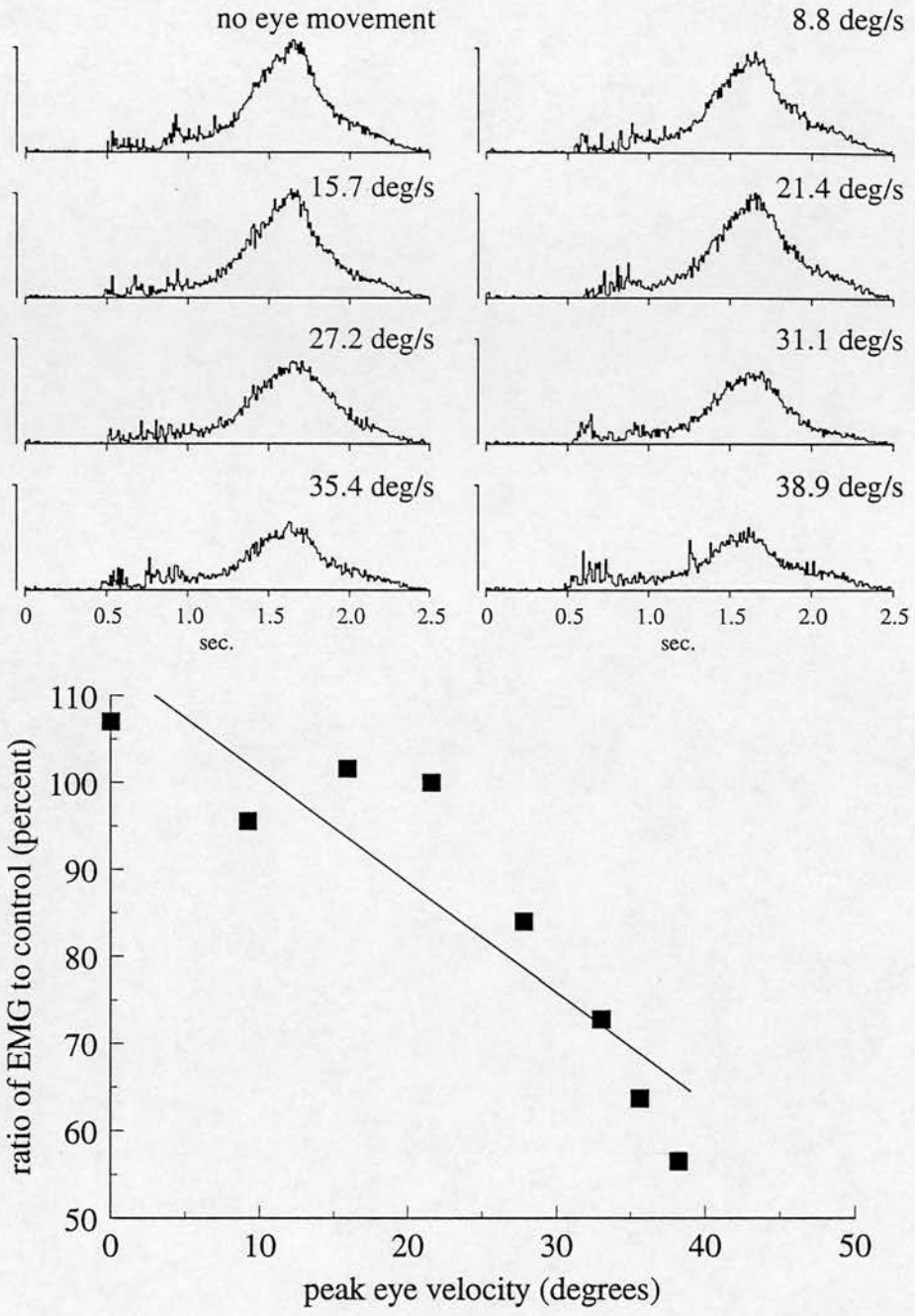


Figure 3.4. Set of eight interleaved cycle histograms (CHSTs) showing the electromyographic activity in the right lateral rectus during sinusoidal, horizontal vestibular stimulation (VEST) alone (top left CHST) and during the artificial VOR (aVOR) with the peak eye velocities marked against the CHSTs. Scale bars for CHSTs, 12 μV. The response at 21.4°s^{-1} represents that during the compensatory aVOR when the imposed head and eye speed are equal but in the opposite direction. The VOR response is greater than at the compensatory aVOR when peak speeds slower than compensatory are imposed and smaller when higher speeds are imposed. The graph in the lower part of the figure shows the ratio of the VOR response of the right *lateral rectus* during imposed movements of the left eye at various velocities, to its VOR response during the compensatory aVOR (peak eye velocity, 21.4°s^{-1}).. The solid line is the linear regression ($r = 0.93$, $P < 0.001$). The slope of the regression line is -1.17, suggesting that the VOR response falls by about 1% for each 1°s^{-1} increase in peak eye velocity.

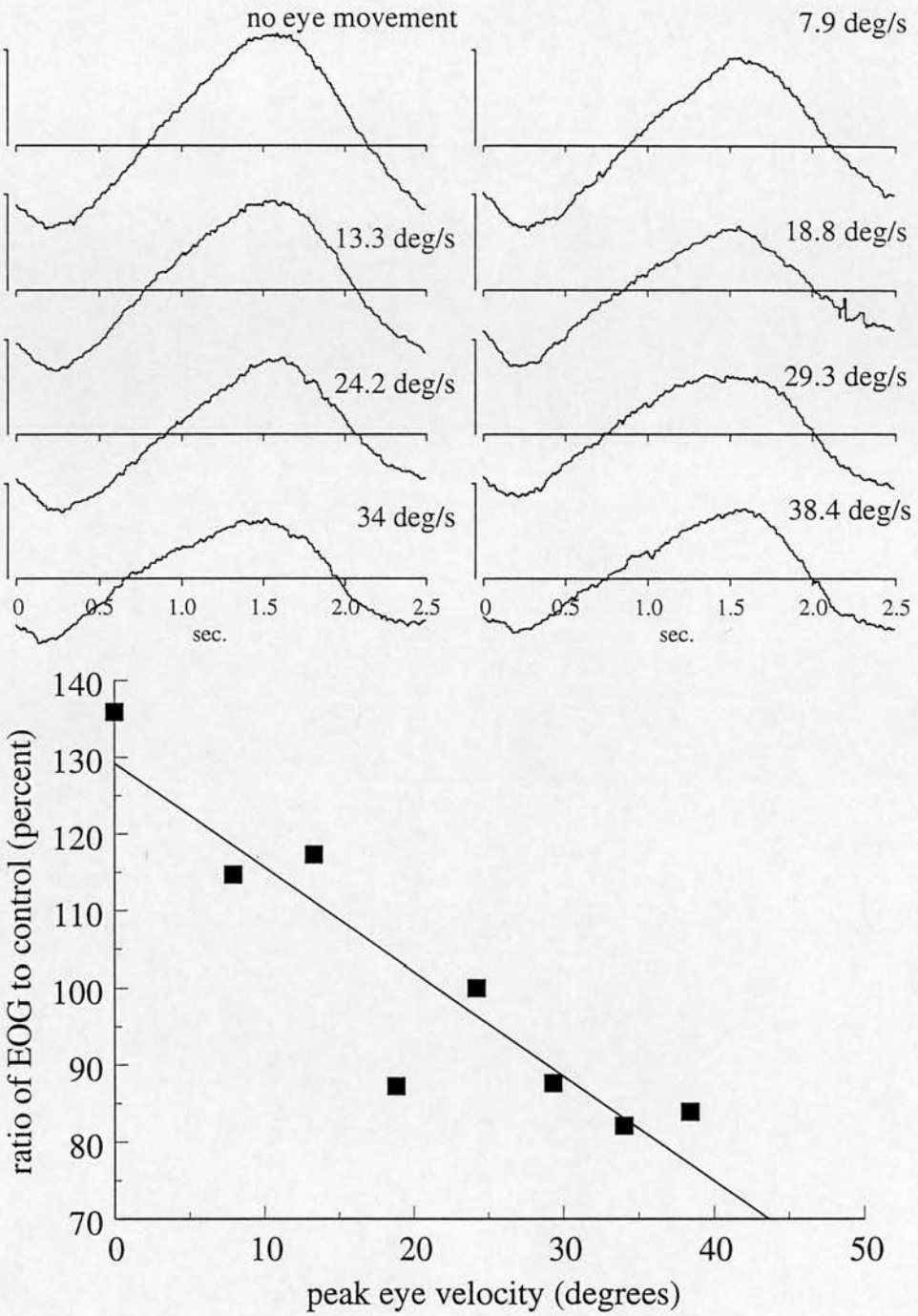


Figure 3.5. Set of eight interleaved cycle histograms (CHSTs) showing the electrooculogram (EOG) recorded from the right eye during sinusoidal, horizontal vestibular stimulation (VEST) alone (top left CHST) and during the artificial VOR (aVOR) with the peak eye velocities marked against the CHSTs. Scale bars for CHSTs, 7.2°. The EOG response at 24.2°s^{-1} represents that during the compensatory aVOR when the imposed head and eye speed are equal but in the opposite direction. The eye movement is greater than at the compensatory aVOR when peak speeds slower than compensatory are imposed and smaller when higher speeds are imposed. The graph in the lower part of the figure is derived in the same manner as that of Figure 3.4. The slope of the regression line (solid line) is -1.33 ($r = 0.92$, $P<0.001$), suggesting that eye movement decreases by about 1% for each 1°s^{-1} increase in peak eye velocity. The VOR gain of the right eye when the left eye was held still was -0.97.

Effect of section of Vo on the VOR response of the lateral rectus

Following section of Vo the effect of imposed eye movement (IEM) of the left eye on the VOR response of the right *lateral rectus* was completely removed. The magnitude and the phase of the VOR response were both unaffected by IEM. This was true both for saccadic imposed eye movement and for the artificial VOR (see Figure 3.6).

Effect of section of Vo on the VOR

Following section of Vo, the effect of the aVOR on the VOR of the right eye which formerly had had a considerable inhibitory effect was completely removed (Figure 3.7). This abolition of the effect of IEM on the VOR of the contralateral eye was seen in four out of four experiments The VOR response of the right eye in response to vestibular stimulation alone, following section of Vo, was similar to that seen before section of Vo. The EOG traces in Figure 3.8 show the similarity between the VOR before and after section of Vo, the difference being a decrease in the gain of the VOR from -0.9 to -0.65. The effect of section of Vo on the VOR response of the left eye was considerably more marked, as can be seen in Figure 3.8. Before the section the left eye showed a consistent VOR with a gain of -0.8, whereas following the section the VOR was much less stable and the VOR gain was both dramatically decreased and much more variable than before the section.

A similar effect was also seen on the stability of the eyes at rest (Figure 3.9). The right eye showed small movements and oscillations both before and after section of Vo, but the left eye, whilst showing similar small movements before the section, was considerably less stable following section of Vo, with far more marked movements and the presence of saccades which were rarely, if ever, seen before Vo section. The effect of Vo section on the stability and VOR of both eyes was seen in four out of four experiments.

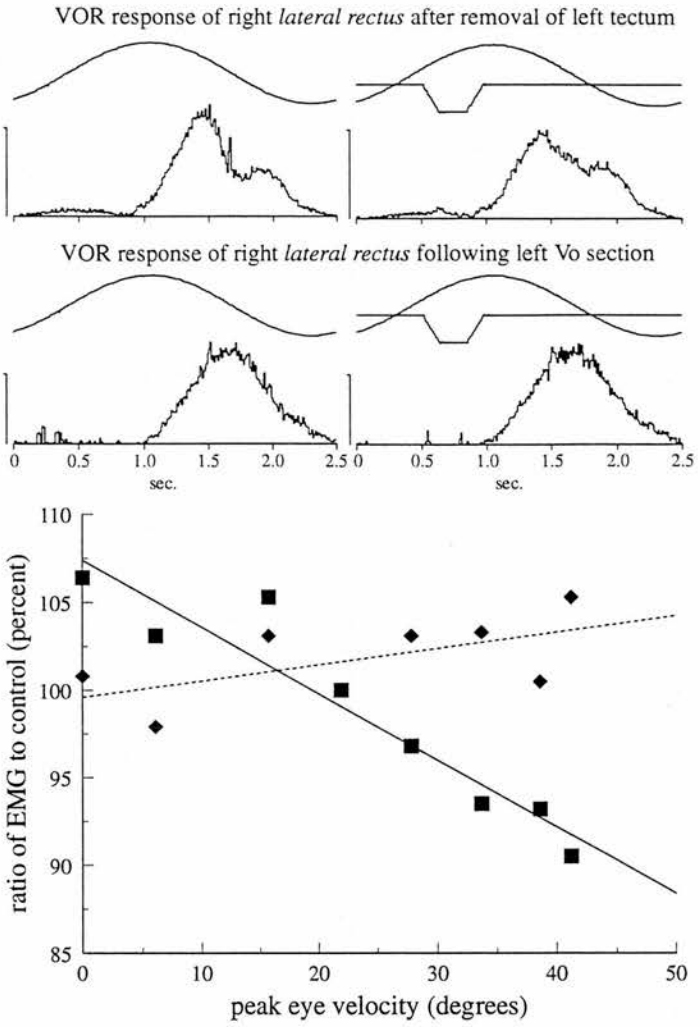


Figure 3.6. Effect of section of the ophthalmic branch of the left trigeminal nerve (Vo) on the inhibitory effect of imposed eye movement of the left eye on the VOR response of the right *lateral rectus* during horizontal vestibular stimulation. The four interleaved cycle histograms (CHSTs) show the effect of saccadic imposed eye movement (sIEM) before (upper two CHSTs) and after (lower two CHSTs) Vo section. The considerable inhibition produced by sIEM imposed 0.5 sec before the start of activity in the right *lateral rectus* following removal of the left tectum is completely abolished by section of Vo. Scale bars for CHSTs, 14 μV. The graph in the lower part of the figure shows linear regression plots (see Figure 3.4) of the results of imposing the artificial VOR (aVOR) with amplitude and velocity errors. Solid line shows the linear regression for the effect of the aVOR on the right *lateral rectus* before section of Vo (solid squares represent data at different peak eye velocities). The slope of the regression line is -0.36 ($r = 0.96$, $P < 0.001$). The dotted line shows the linear regression plot (diamonds show data points) for the experiment performed following section of Vo. The slope of the regression line is 0.08 ($r = 0.49$, $P > 0.1$); the effect of the aVOR on the VOR response of the right *lateral rectus* has been removed by Vo section.

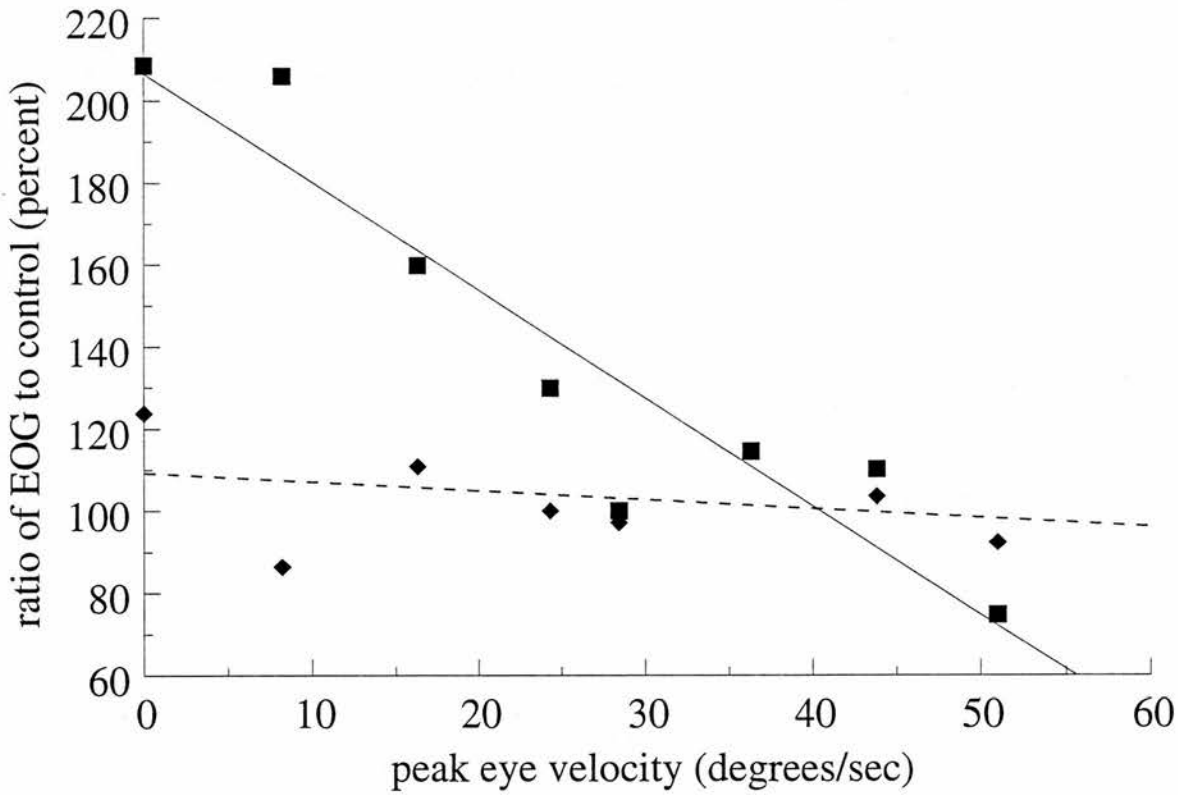


Figure 3.7. Graph to show the effects of section of the ophthalmic branch of the left trigeminal nerve (Vo) on the EOG of the right eye whilst an artificial VOR (aVOR) with velocity and amplitude errors was imposed on the left eye. Graph is constructed as described in Figure 3.4. Solid line shows the linear regression for the experiment performed before section of Vo. The slope of the regression line is -2.77 ($r = 0.94$, $P < 0.001$). The dotted line shows the regression line from the experiment performed after Vo section which has a slope of -0.23 ($r = 0.31$, $P > 0.1$). The VOR gain of the right eye was approximately -1.0 both before and after left Vo section.

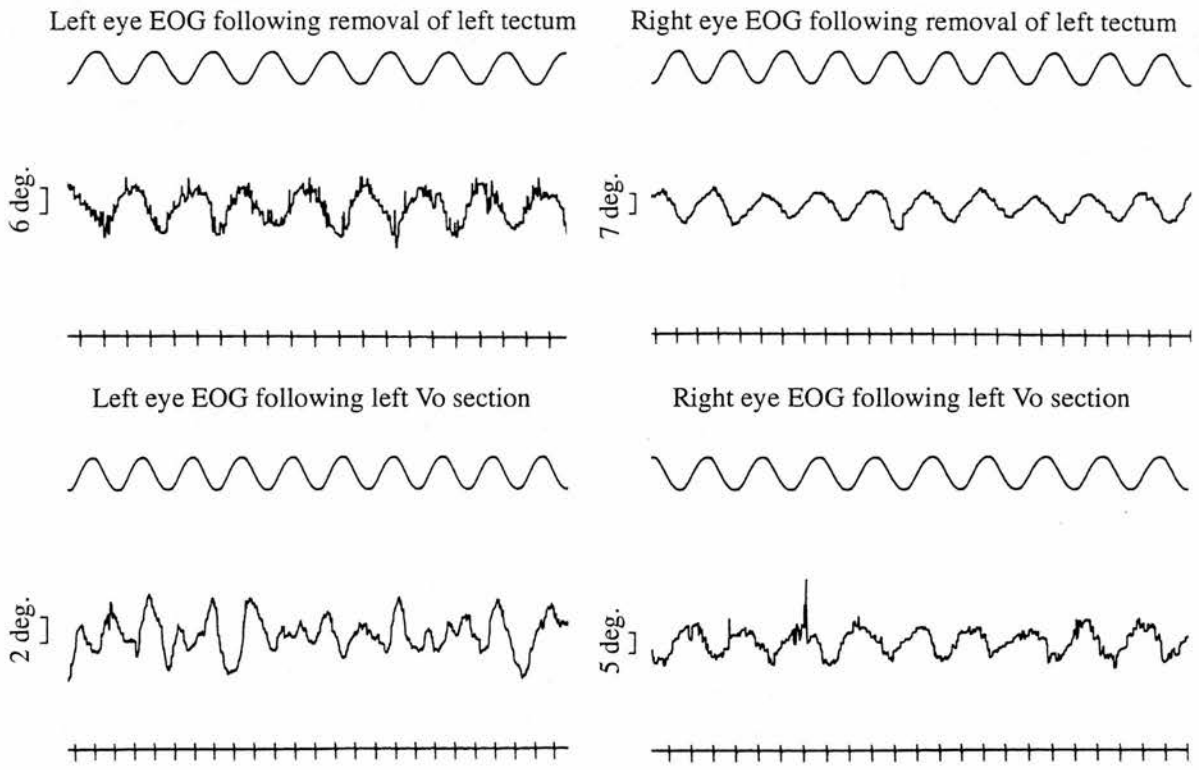


Figure 3.8. Scanned pen recorder traces of vestibular table movement (upper record in each trace), EOG (middle record) and time (lower record, 1 sec ticks) during horizontal vestibular stimulation (0.4 Hz, ± 8°). EOG traces show the VOR in the left and right eyes following removal of the left tectum (upper two traces) and after subsequent section of the ophthalmic branch of the trigeminal nerve (Vo). The left eye is dramatically affected by Vo section with a markedly disrupted VOR, whereas the right eye shows little change in the size or shape of its VOR.

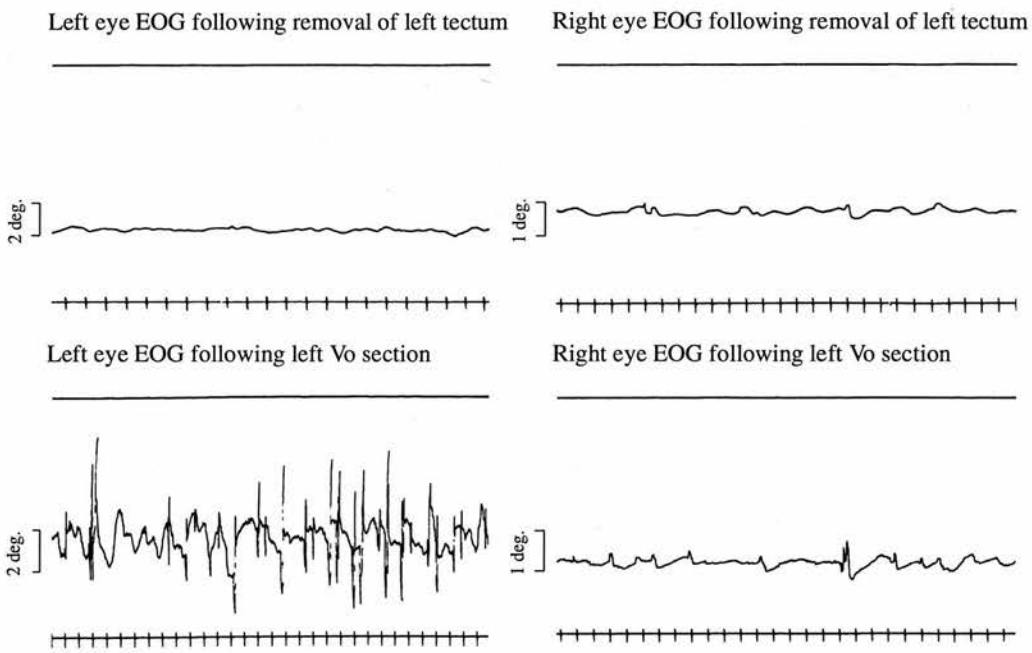


Figure 3.9. Scanned pen recorder traces showing the stability of the eye at rest. The vestibular table was held still (upper record in each trace) and the EOG signal (middle record) recorded. The left and right eyes both show very little movement in the absence of vestibular stimulation following removal of the left tectum (upper two traces). After section of the ophthalmic branch of the trigeminal nerve (Vo), the left eye showed considerable movement with a number of fast phase oscillations. The right eye was little changed following left Vo section.

3.4 DISCUSSION

The results of the experiments on the right *lateral rectus* muscle confirm and extend those of Knox and Donaldson (1991). Of particular interest is the strongly inhibitory effect of saccadic imposed eye movement (sIEM) of the left eye on the right *lateral rectus* a considerable time before activity occurred in this muscle. This long latency between the start of sIEM and the start of activity in the right *lateral rectus* is appreciably different from the effect of sIEM on the dorsal neck musculature as described in Chapter 2. However, there is a similarity between these effects. sIEM produced the largest inhibitory effect in the right *lateral rectus* when the sIEM occurred at such a time that the contracting left *lateral rectus* was moved in the opposite direction; in effect a contracting muscle was stretched. It is interesting to note that this effect appeared to be specific to stretch of the *lateral rectus* muscle, because sIEM that would have stretched the contracting left *medial rectus* (i.e. sIEM occurring at the same time as activity in the right *lateral rectus*) did not produce an inhibition in the VOR response of the right *lateral rectus* (see Figure 3.2, delay = 100 and 115 msec, orientation = 90°).

The effect of the artificial VOR with velocity and amplitude errors on the VOR response of the right *lateral rectus* had already been demonstrated by Knox and and Donaldson (1991), but an analysis of covariance had not been undertaken for different experiments. The present study did perform this test for 5 experiments and found a single group, homogeneous in slope. The slope of this group was close to -1, corresponding to a 1% decrease in the VOR response of the right *lateral rectus* for each 1°/sec increase in the peak eye velocity of the aVOR. This is very similar to the value gained from pooled regression data on the effect of the aVOR with velocity and amplitude errors on vestibularly-modulated single units in the pigeon brainstem (Donaldson and Knox, 1993), on the movement of the globe itself (Knox and Donaldson, 1993 and see later) and on the VCR activity of dorsal neck muscles (see Chapter 2) during horizontal vestibular stimulation. The consistency of the effect of the aVOR on these various levels of the vestibular gaze control system suggests that IEM affects a fundamental part of this system, possibly the activity of vestibular neurones which, in turn, affect the output of both the vestibuloocular and vestibulocollic reflexes in systematic and predictable ways.

The removal of any effect of imposed eye movement on the *lateral rectus* following section of the ophthalmic branch of the trigeminal nerve (Vo) is in agreement with the effect of Vo section on dorsal neck muscles (see Chapter 2) and

provides further evidence that the effective signal produced by imposed eye movement originates in extraocular muscle proprioceptors (EOM) and passes through Vo towards the brainstem (see Chapter 4).

Studying the movements of both eyes with the electrooculogram and the effect of section of Vo provided further evidence about the importance of EOM proprioception in the control of eye movements. It is remarkable how closely the effects of unilateral EOM deafferentation in the decerebrate pigeon match those seen following similar deafferentation in mammals (see Fiorentini and Maffei, 1977; Kashii et al, 1989 and Kimura et al, 1991). The instability of the deafferented eye and the low and inconsistent VOR gain strongly imply that EOM proprioceptive afferent signals play a major role in the control of eye movements. The results also show that, at least in the pigeon, the effects of deafferentation are effectively immediate and not the result of long-term parametric changes in the calibration of internal copies of eye position, long a refuge of the desperate proponent of corollary discharge! Dramatic effects following Vo section are additional proof that Vo section removes a functionally significant signal rather than relatively non-specific noci- or mechanoreceptive afferent signals that have been suggested to be the source of the observed effects of imposed and passive eye movement.

CHAPTER 4. LOCALIZATION OF PIGEON EXTRAOCULAR MUSCLE AFFERENT NEURONES AND THEIR BRAINSTEM TERMINATIONS IDENTIFIED BY TRANSGANGLIONIC TRANSPORT OF WHEAT GERM AGGLUTININ-CONJUGATED HORSERADISH PEROXIDASE

4.1 INTRODUCTION

The numerous histochemical and electrophysiological studies on the site(s) of the first-order neurones of extraocular muscle (EOM) proprioceptors and their brainstem terminations have been described in considerable detail in Chapter 1 and will only be summarised here. Briefly, in all species so far examined, first-order sensory neurones, presumed to be innervating EOM proprioceptors and other sensory receptors, have been localized to the ophthalmic subdivision of the trigeminal ganglion. There is some discussion in studies in the cat that a few first-order neurones are also located in the mesencephalic trigeminal nucleus, although this appears unlikely. All of the histochemical studies have utilised the anatomical tracer horseradish peroxidase (HRP) in varying concentrations and volumes as detailed in Chapter 1. Further anatomical studies using the anterograde tracing agent, wheatgerm agglutinin conjugated to HRP (WGA-HRP) have shown the presence of terminals in the spinal trigeminal nucleus. The precise location of these terminals varies from study to study, but the consensus appears to be that the largest concentration of terminals is found in the pars interpolaris division of the nucleus. These studies are in agreement with a number of electrophysiological studies which have recorded short latency responses to EOM stretch in the spinal trigeminal nucleus and the emerging fibres of the trigeminal nerve in the brainstem (Manni et al, 1972; Cooper, Daniel and Whitteridge, 1953a, b; Fillenz, 1955).

The only exception to these findings is the study of Eden and co-workers (1982) in the pigeon. They reported finding labelled first-order proprioceptive neurones in the pigeon brainstem following injection of high concentrations of HRP into individual EOM. They did not investigate whether there were also labelled cells within the trigeminal ganglion and presented no evidence of terminal labelling in the brainstem.

This surprising result appears to imply that the neural wiring of EOM proprioceptors in the pigeon and possibly all birds (for no other species have been investigated to date) differs greatly from that observed in mammals. This is surprising, since Eden et al quote the work of Kappers et al (1967) on the similarity of the organization of the sensory trigeminal nuclei in the bird and in many mammals. Furthermore, Eden et al's (1982) study weakens the comparison between

the effects of stimulating EOM proprioceptors in the pigeon and the effects of EOM proprioceptors in mammals, including Man. This runs contrary to what might otherwise have been thought (Donaldson and Knox, 1990a).

The present study of the innervation of pigeon EOM sensory and motor-neurones and the possible brainstem terminations of these sensory neurones was initially carried out to repeat Eden et al's (1982) study in order to test their surprising results.

4.2 METHODS

The central projections of pigeon (*Columba livia*) extraocular muscle afferent neurones were determined by the technique of transganglionic transport of wheat germ agglutinin-conjugated horseradish peroxidase (WGA/HRP) and retrograde transport of horseradish peroxidase (HRP) (Mesulam, 1978; Rosene and Mesulam, 1978; Porter, 1986 and personal communication). Experiments were conducted in 17 adult (older than ten weeks) pigeons. Each pigeon was decerebrated under ether anaesthesia as described in Chapter 2 and then secured in a stereotaxic headholder. The pigeon globe is flattened (unlike the spherical mammalian globe) and the extraocular muscles (EOMs) lie behind the ridge of the globe; therefore, the EOM could only be exposed following considerable dissection. The superior rectus (SR) and superior oblique (SO) muscles were exposed by reflecting the skin above the eye downwards and cutting through the fascia and connective tissue joining the skin to the skull and eye. The cartilage and bone of the superior wall of the orbit were removed with bone rongeurs, and overlying fat and connective tissue were also removed. The medial, lateral and inferior recti (MR, LR, IR) and inferior oblique (IO) muscles were exposed following removal of the upper and lower eyelids and surrounding skin. The globe was then pierced and deflated by removing a portion of the vitreous body (humour) with a syringe inserted into the globe. The globe was then rotated both upwards and medially allowing access to the LR, IR and IO muscles, and downwards and laterally to facilitate access to the MR muscle.

Selected EOMs were injected with 2 - 5 µl of a mixture of 1% (w/v) WGA/HRP (Sigma) and 5% HRP (Sigma) or, in later experiments, 2% (w/v) WGA/HRP and 10% (w/v) HRP in sterile water through a Hirumo microsyringe equipped with a 27-gauge needle. Details of the muscles injected are presented in Table 1. Individual EOM were injected over a period of 5 minutes, and the needle was then left in place for a further 5 minutes following injection to reduce the leakage of tracer from the muscles. A single needle penetration was used to further reduce the possibility of leakage.

Pigeons were then oscillated on a vestibular turntable for a number of hours to stimulate activity within the EOM and, following a postoperative survival time of 16-30 hours, were terminally anaesthetised and the brain was fixed by transcardiac carotid catheterization and perfusion (Eden and Correia, 1987). Prior to perfusion, 20% heparin sodium (5,000 IU, in 0.09% saline, pH 7.4) was administered intracardially. Intracarotid catheters were inserted through the left ventricle into the left and right brachiocephalic arteries and advanced approximately 1 cm into the

carotid arteries where they were clamped with artery clips (method taken from Eden and Correia, 1987). Pump perfusions were initiated with 1 litre of physiological saline (pH 7.4, room temperature) containing 1% sodium nitrite, followed by 1 litre of fixative solution containing 1% paraformaldehyde-1.25% glutaraldehyde in 0.1M phosphate buffer (pH 7.4, room temperature). The pump rate was set at about 50 ml/min for the saline flush and the first 500 ml of fixative solution, and at a slower rate (20 - 25 ml/min) for the remaining 500 ml of fixative so that the total perfusion time was approximately 60 minutes. The remaining brain and Trigeminal ganglion from the side of EOM injection were removed and immersed in 100 ml of perfusion cold (4°C) fixative for an additional hour and then placed overnight in cold 0.1 M phosphate buffer (pH 7.4), containing 10% sucrose. Serial 50-μm sections of brainstem and ganglion were cut with a freezing microtome and processed for the histochemical demonstration of HRP using a modification of the tetramethylbenzidine chromogen protocol (Mesulam, 1978; Porter, 1986, personal communication). Sections were washed in 3 changes of distilled water (5-10 min/change) and preincubated in the dark with constant agitation in media containing 0.005% 3,3',5,5'-tetramethylbenzidine, 0.015% sodium nitroferricyanide, and 2.5% ethanol in acetate buffer (pH 3.3) at room temperature. Following a 20-minute pre-incubation, hydrogen peroxide was added to the media to achieve a concentration of 0.3% (brainstem sections) or 0.15% (ganglion) and sections were incubated, still in darkness, for a further 15 minutes. Sections were then rinsed six times in cold postreaction and rinsing solution (5 ml of acetate buffer (pH 3.3) in 95 ml of distilled water) over half an hour, mounted on slides coated with albumen, and counterstained with 1% neutral red for light microscopic examination by using brightfield and polarized light illumination. The distribution of HRP-labelled axon terminals was charted from sections with reference to nuclear boundaries by using a microscope equipped with a drawing attachment. Brainstem nuclear designations were based upon the atlas of Karten and Hodos (1967) and with reference to the studies of head muscle innervation by Wild and Zeigler (1980), the trigeminal system in the pigeon (Dubbeldam and Karten, 1978) and of EOM innervation by Evinger (1988).

In two experiments the ophthalmic branch of the trigeminal nerve was sectioned and a two millimetre portion removed, following exactly the same procedure as described in Chapter two, before injection of WGA-HRP and HRP into selected EOM.

4.3 RESULTS

Labelling of cell somata

Brainstem sections extending caudally from the rostral pole of the oculomotor nucleus to approximately the second cervical spinal cord segment were examined for the presence of retrogradely labelled cell somata and anterogradely labelled afferent terminals. Injections of wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP) and horseradish peroxidase (HRP) into combinations of all six extraocular muscles (EOM) of one side (see Table 1), labelled motorneurones in appropriate subdivisions of the contralateral oculomotor (superior rectus), ipsilateral oculomotor (medial and inferior recti and inferior oblique), contralateral trochlear (superior oblique) and ipsilateral abducens (lateral rectus) nuclei (see Evinger, 1988 for a description of pigeon oculomotor motorneuron representation) (see Figure 4.1). The distinct pattern of distribution of labelled motorneurones served to confirm that the desired muscles were injected and to control for spread of tracer from the injection site. In one case, leakage of tracer was indicated by the presence of some poorly-filled neurones in the depressor palpebrae inferioris muscle subdivision of the ipsilateral trigeminal motor nucleus (Wild and Zeigler, 1980). No such evidence of leakage of tracer was evident in the remaining birds.

Heavily labelled multipolar neurones were also noted to cluster consistently in the same small ventrolateral region of the pons (Figure 4.3). Two distinct groups of labelled neurones were obvious within this cluster based on shape and size. Larger and more densely labelled, multipolar neurones were located dorsolateral and rostral to considerably smaller, triangular and elongated, more sparsely labelled neurones. The similarity to the presumed "medullary proprioceptive neurones" of Eden et al (1982) was very notable. However, whereas Eden et al located these labelled neurones in "the same small ventromedial segment of the ipsilateral nucleus descendens nervi trigemini (TTD)" the cells labelled in the present experiments lay just above the superior olive and near, but ventral, to the spinal trigeminal nucleus or TTD. The position and appearance of these labelled cells was more suggestive of motorneurones than potential primary afferent cell bodies which are usually pseudounipolar with no axons or dendritic tree obvious. The similarity between both the position and appearance of these labelled cells and the neurones labelled in the accessory abducens nucleus following injection of HRP into the pyramidalis and quadratus muscles of the chick (Labandeira-Garcia et al, 1987) was striking. The axons of the labelled cells headed in a dorsomedial rostral direction towards the

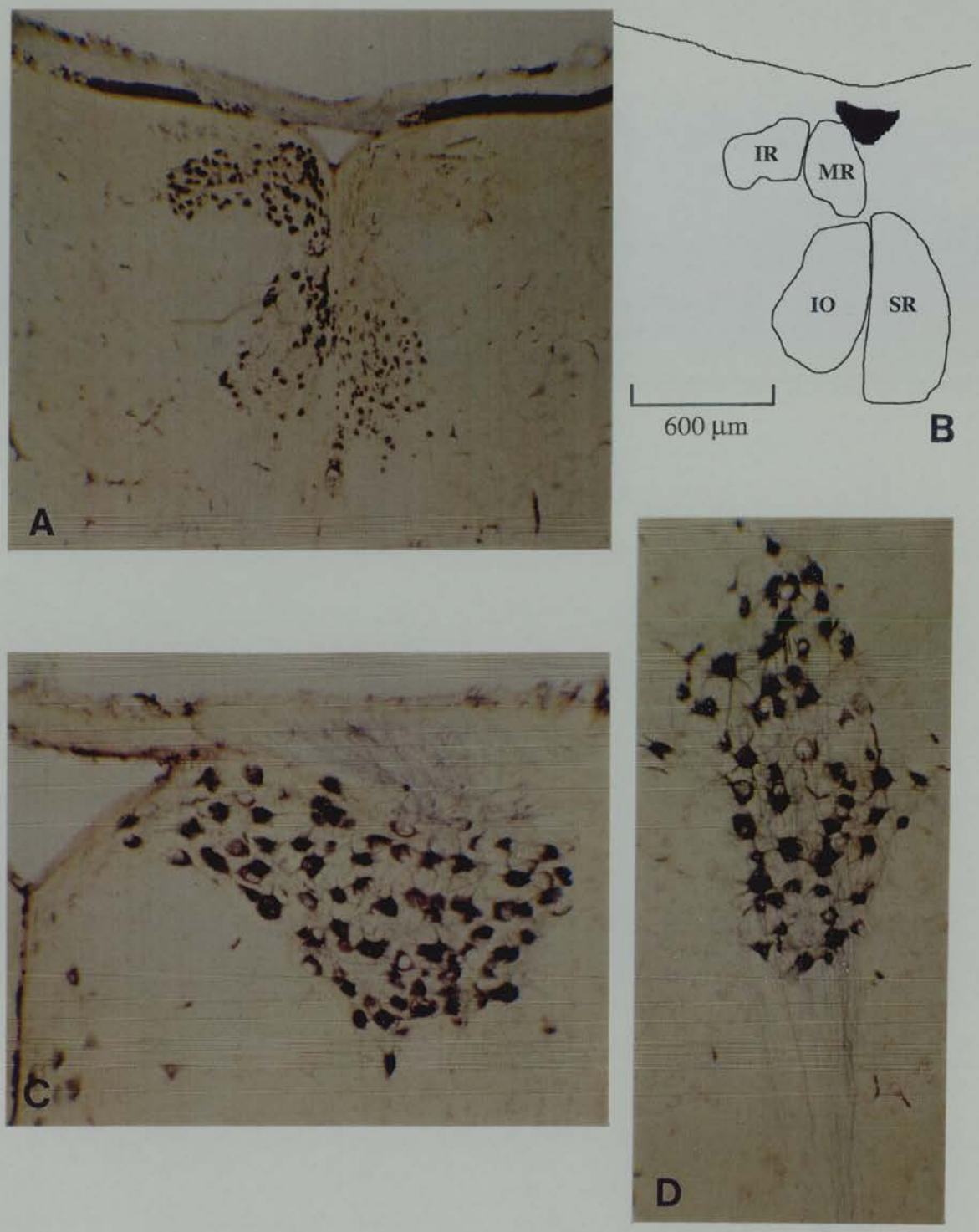


Figure 4.1 Photomicrographs of labelled motorneurone somata following injection of WGA-HRP and HRP into all six extraocular muscles (EOM) of the pigeon. Retrogradely labelled motorneurones were found bilaterally in the oculomotor nucleus (A), within the contralateral trochlear nucleus (C) and within the ipsilateral abducens nucleus (D). (B), scanned image of oculomotor nucleus photomicrograph indicates arrangement of motorneurone subgroups within this nucleus corresponding to retrograde transport of WGA-HRP and HRP from the medial rectus (MR), superior rectus (SR), inferior rectus (IR) and inferior oblique (IO) muscles. The patterned distribution of labelled motorneurones corresponds to previously reported representations of motorneurone subgroups (Evinger, 1982). A, D × 100; B, C × 40.

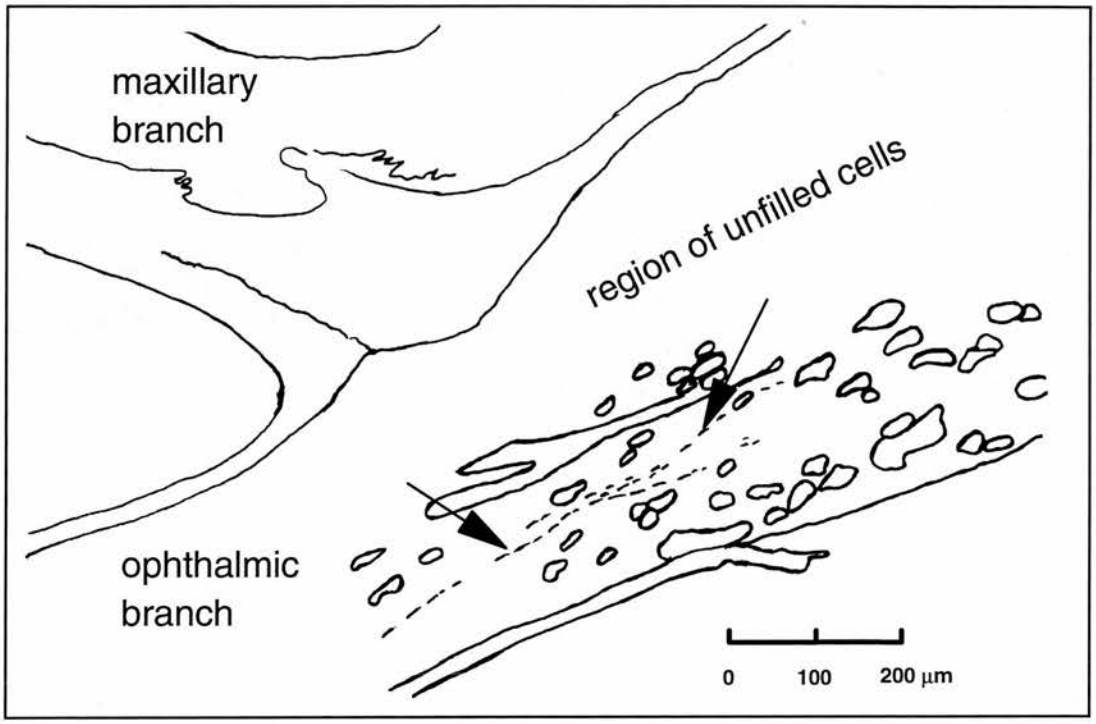


Figure 4.2 *verso* Diagram of photomicrograph A (opposite) to show the distribution of labelled and unlabelled afferent neurones and an example of the labelled fibres (arrows) coursing through the ophthalmic subdivision of the trigeminal nerve to the labelled cells within the trigeminal ganglion.

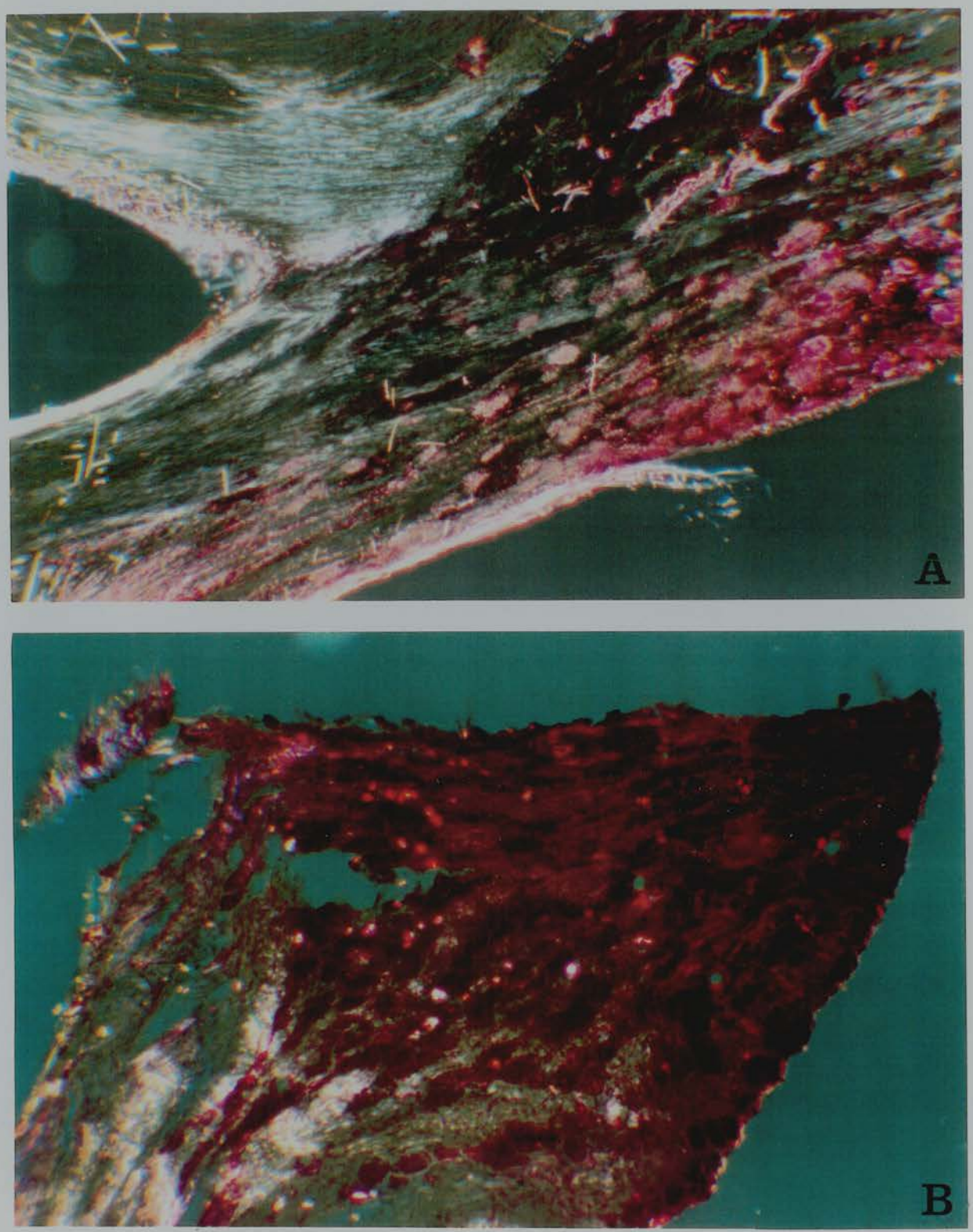


Figure 4.2. Photomicrographs taken with polarized light illumination of the trigeminal ganglion following injection of WGA/HRP and HRP into the extraocular muscles. (A) showing labelled pseudounipolar (presumed afferent) neurones in a restricted portion of the ganglion. (B) showing the absence of any similarly labelled neurones following section of the ophthalmic branch of the trigeminal nerve prior to tracer injection. × 100.

principal abducens nucleus and then turned to join the axons of abducens motorneurones as they headed ventromedially to form the abducens nerve. This is the path taken by accessory abducens motorneurones in the chick and other animals which strongly suggests that the labelled neurones in this study were also accessory abducens motorneurones. That this possibility was indeed the case was confirmed by an experiment in which 2 µl of WGA/HRP were injected into the quadratus muscle, which inserts inferior to the superior oblique and superior rectus muscles. Labelled motorneurones were found in exactly the same ventrolateral position and in similar numbers to the earlier EOM afferent labelling experiments (Figure 4.3). Labelled axons were seen coursing dorsomedially towards the abducens nucleus and then ventrally within the abducens nerve without the presence of labelled abducens motorneurones. In fact, no labelled motorneurones were found in any of the oculomotor nuclei; no terminal labelling was found in the ipsilateral spinal trigeminal or external cuneate nucleus (see later) and a total of three labelled pseudounipolar neuronal cell somata were found in the ipsilateral trigeminal ganglion.

Labelled pseudounipolar neuronal cell somata, presumptive EOM afferent neurones, were found in a restricted portion of the ipsilateral trigeminal ganglion (Figure 4.2). The trigeminal ganglion is known to contain a precise somatotopic representation of ophthalmic, maxillary and mandibular facial regions in most animals, including the pigeon (Zeigler et al, 1975). Whilst it was difficult to ensure that the trigeminal ganglion was sectioned in the same plane in each experiment, labelled cell somata were always restricted to a small portion of the ganglion, presumably the ophthalmic subdivision. In one experiment, labelled axons could be seen coursing along the ophthalmic branch of the trigeminal nerve and entering the ganglion, terminating in the same region as the labelled cells.

The pigeon has a far more enclosed, bony orbit than the cat, which may partly explain the absence of any labelled cells in the trigeminal mesencephalic nucleus in all of the 16 tracer experiments, even when there was evidence of spread to extraorbital muscles, since it is believed that labelled cells in the trigeminal mesencephalic nucleus of the cat represent spread to jaw muscle proprioceptors (Porter and Donaldson, 1991).

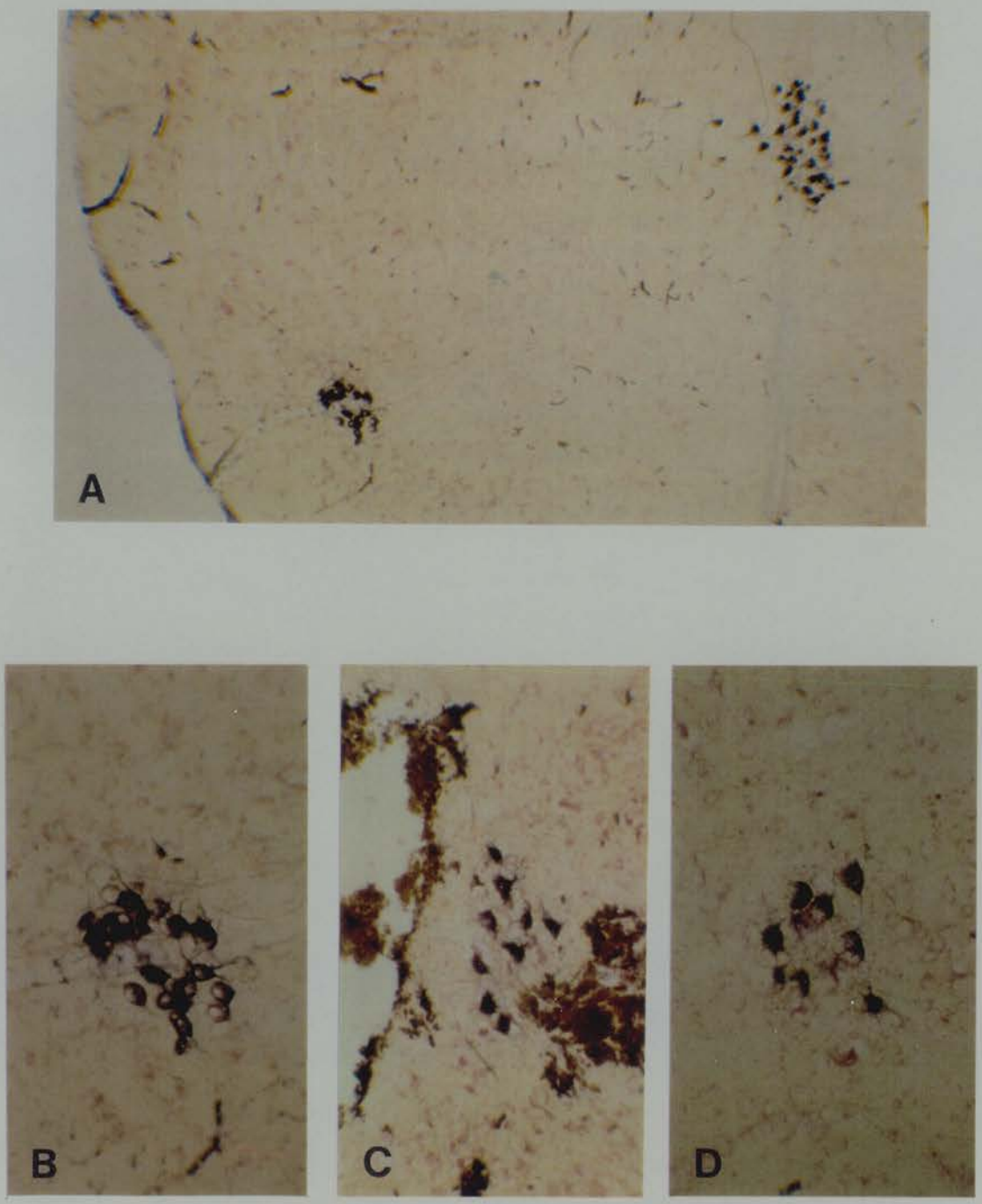


Figure 4.3. Photomicrographs of labelled motorneurone somata within the accessory abducens nucleus in the ventrolateral pons. (A), labelled motorneurones in the accessory abducens nucleus and the abducens nucleus following injection of WGA/HRP and HRP into the superior rectus (SR) and superior oblique (SO) muscles. (B), higher power view of labelled accessory abducens neuronal somata from (A). (C), labelled accessory abducens motorneurones following section of the ophthalmic branch of the trigeminal nerve and subsequent injection of SR and SO. (D), labelled accessory abducens motorneurones after injection of a small volume (1 µl) of WGA/HRP and HRP directly into the quadratus muscle, deep to SO and SR. A × 40; B-D × 100.

TABLE 1.

Expt. (survival time / hrs.)	EOM injected	Volume of WGA/HRP + HRP injected (µl/EOM)	Labelled oculomotor motoneurones	Labelled accessory abducens motoneurones	Terminal labelling (cuneate externus)	Labelled cell somata in trigeminal ganglion
6 (22)	S.R. & S.O.	2 × 5 µl	✓✓	✓✓	✓	✓✓
7 (31)	S.R. & S.O.	2 × 5 µl	✓✓	✓✓	✓✓	✓
9* (18)	S.R. & S.O.	2 × 5 µl	✓✓	✓✓	•	•
11 (18)	S.O.,S.R.,I.R., I.O. & L.R.	5 × 5 µl	✓✓✓✓✓	✓✓	✓	✓✓
13 (15)	S.O.,S.R., I.O. & L.R.	4 × 3 µl + 1 × 5 µl	✓✓✓✓	✓✓	✓✓✓	✓✓
14 (24)	S.O.,S.R.,I.R., I.O. & L.R.	5 × 3 µl	✓✓✓✓✓	✓✓	✓✓✓	✓✓
15 (24)	S.O.,S.R.,I.R., I.O. & L.R.	5 × 1.5 µl	✓✓✓✓✓	✓✓	✓✓	✓✓
16 (30)	S.O.,S.R.,I.R. & I.O.	4 × 2.5 µl	✓✓✓✓	✓✓	✓	✓✓

* - ophthalmic branch (Vo) of the trigeminal nerve sectioned

Labelling of afferent terminals

Anterogradely labelled axons were observed to enter the brainstem within the trigeminal nerve and descend within the ventral portion of the lateral spinal trigeminal tract. No terminal labelling was apparent in any of the experiments within the ipsilateral spinal trigeminal nucleus (TTD) (Figure 4.5). Labelled axons were noted to descend into the caudal brainstem in the lateral TTD or lateral spinal trigeminal tract. Terminal labelling was present within the ipsilateral external cuneate nucleus, dorsolateral to the spinal trigeminal nucleus (TTD). The extent of the terminal labelling was limited (maximum of approximately 600-800 µm) as was seen for studies in the cat and monkey (Porter and Donaldson, 1991; Porter, 1986). The extent, and even the presence, of terminal label appeared to be closely related to the number of EOM injected with tracer and to the volume of tracer injected into individual EOM (Table 1). Injections into multiple EOM were necessary to produce significant terminal labelling (Figure 4.4). The survival time also appeared to affect the presence of terminal label, particularly when few EOM and small volumes of tracer were injected. Terminal labelling was not observed in other divisions of the trigeminal sensory complex or in any other brainstem nuclei (Figure 4.5).

Effect of section of Vo

Section and removal of a 2-3 mm portion of the ophthalmic branch of the trigeminal nerve before injection of WGA-HRP and HRP into the EOM prevented all labelling of cell somata in the trigeminal ganglion, and no terminal labelling was observed (Figure 4.2). Labelled motorneurones were present in the relevant oculomotor nuclei and labelled multipolar neurones were present in the ventrolateral pons, the presumptive accessory abducens nucleus (Figure 4.3).

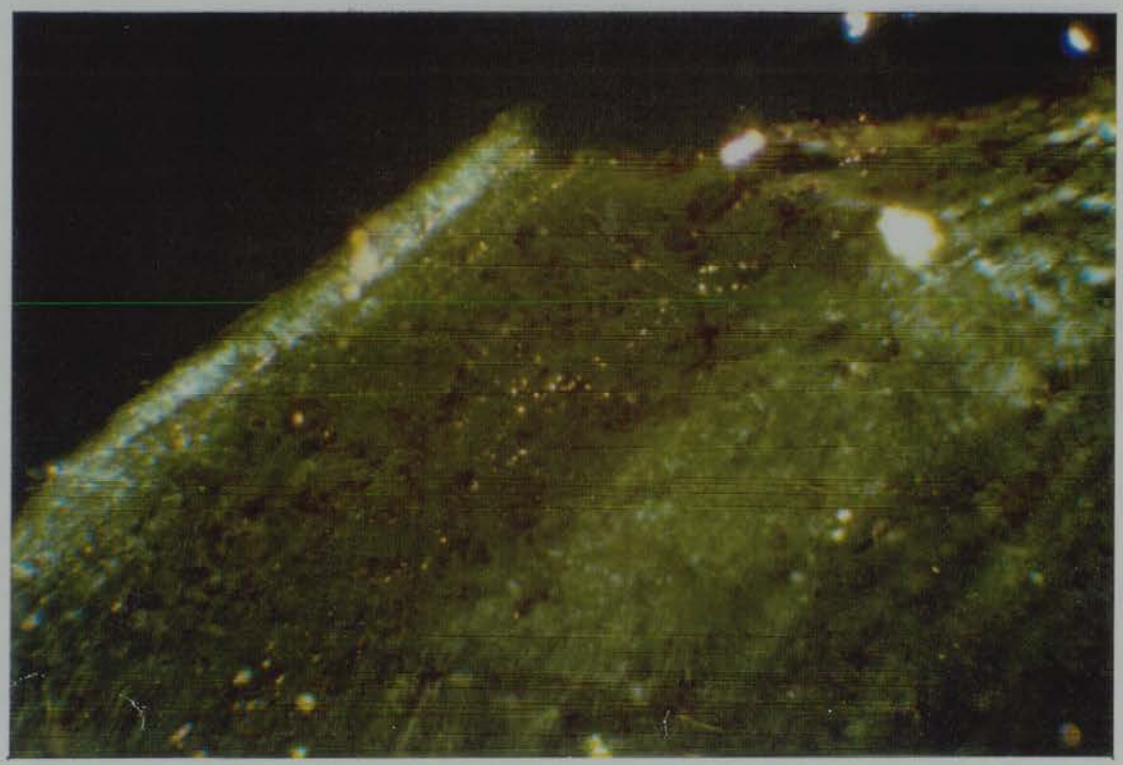


Figure 4.4. Photomicrograph of anterograde terminal label in the ipsilateral external cuneate nucleus (CuE). With polarized light illumination, the anterogradely labelled terminals are pink dots against the dark background. A clear cluster of label is seen within the CuE, the medial border of the nucleus visible as a lighter band of tissue. × 200.

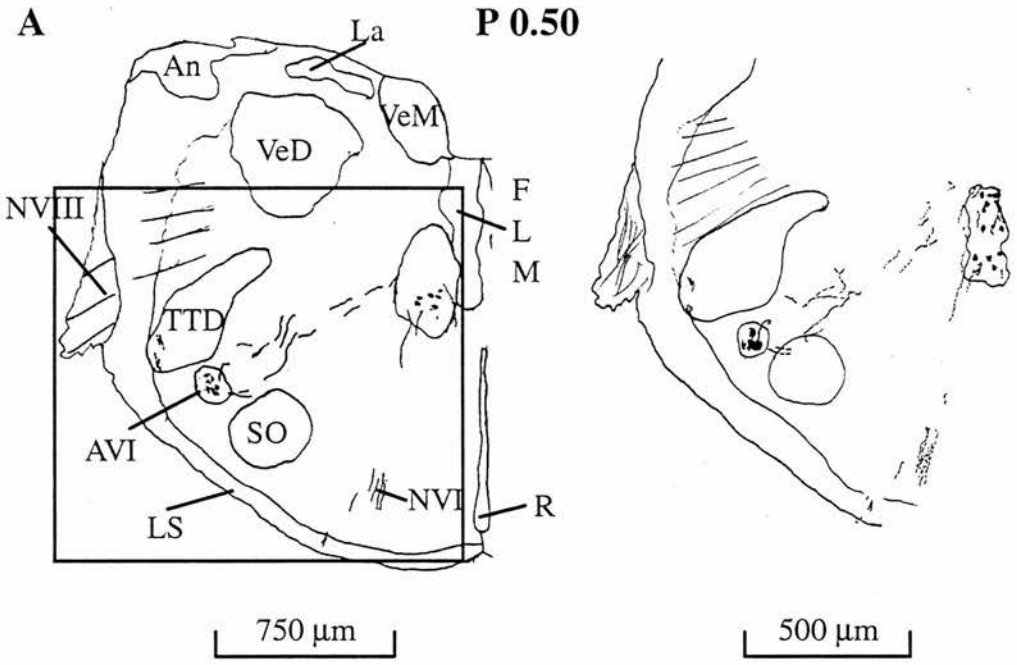
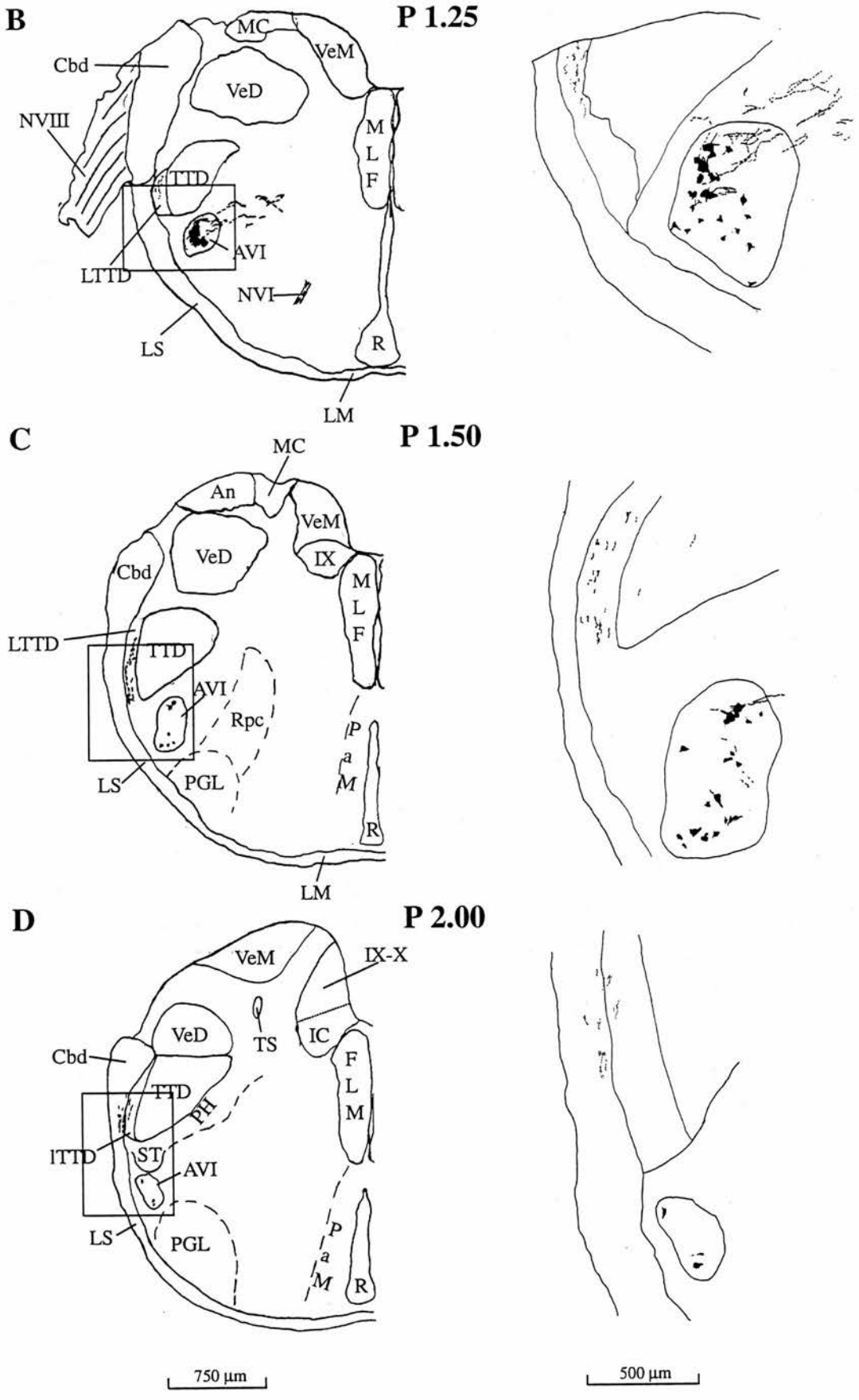


Figure 4.5. Charting of the location of WGA/HRP reaction product within the brainstem following injections of the superior oblique, superior rectus, lateral rectus, inferior rectus and inferior oblique muscles. Coronal sections proceed from rostral (A) through caudal (G). Stippling depicts terminal fields of extraocular muscle afferent neurones in the external cuneate nucleus (sections D-G). Stippled fibres represent axons of passage in the lateral spinal trigeminal tract (lTTD). Sections also show retrogradely labelled lateral rectus motorneurones in the abducens nucleus (VI) (section A) and retrogradely labelled quadratus motorneurones in the accessory abducens nucleus (AVI)(sections A-C) along with axons coursing to the abducens nucleus and then along with axons from abducens motorneurones proper in the sixth nerve (NVI). Numbers located at the right-hand edge of each section indicate approximate distance, in millimetres, in the anterior-posterior plane from the atlas of Karten and Hodos (1967).

Abbreviations

AN	Nucleus angularis
AVI	Accessory abducens nucleus
Cbd	Tractus spinocerebellaris dorsalis
CC	Central canal
CE	Nucleus cuneatus externus
CG	Nuclei gracilis et cuneatus
FLM	Fasciculus longitudinalis medialis
IO	Inferior olive
IC	Nucleus intercalatus
IM	Nucleus intermedius
La	Nucleus laminaris
LM	Lemniscus medialis
LS	Lemniscus spinalis
lTTD	lateral tractus descendens nervi trigemini (lateral spinal trigeminal tract)
MC	Nucleus magnocellularis
NVI	Abducens nerve
NVIII	Vestibulococchlear nerve
PGL	Nucleus paragigantocellularis lateralis
PH	Plexus of Horsley
R	Raphe nucleus
RL	Nucleus reticularis lateralis
Rpc	Nucleus reticularis gigantocellularis
S	Nucleus solitarius
SG	Substantia gelatinosa Rolandi
SO	Superior olive
ST	Subtrigeminal nucleus
TS	Tractus solitarius
TTD	Nucleus et tractus descendens nervi trigemini (spinal trigeminal nucleus and tract)
VeD	Nucleus vestibularis descendens
VeL	Nucleus vestibularis lateralis
VeM	Nucleus vestibularis medialis
VI	Abducens nucleus
IX	Glossopharyngeal nucleus
X	Dorsal motor nucleus of the Vagus
XII	Hypoglossal nucleus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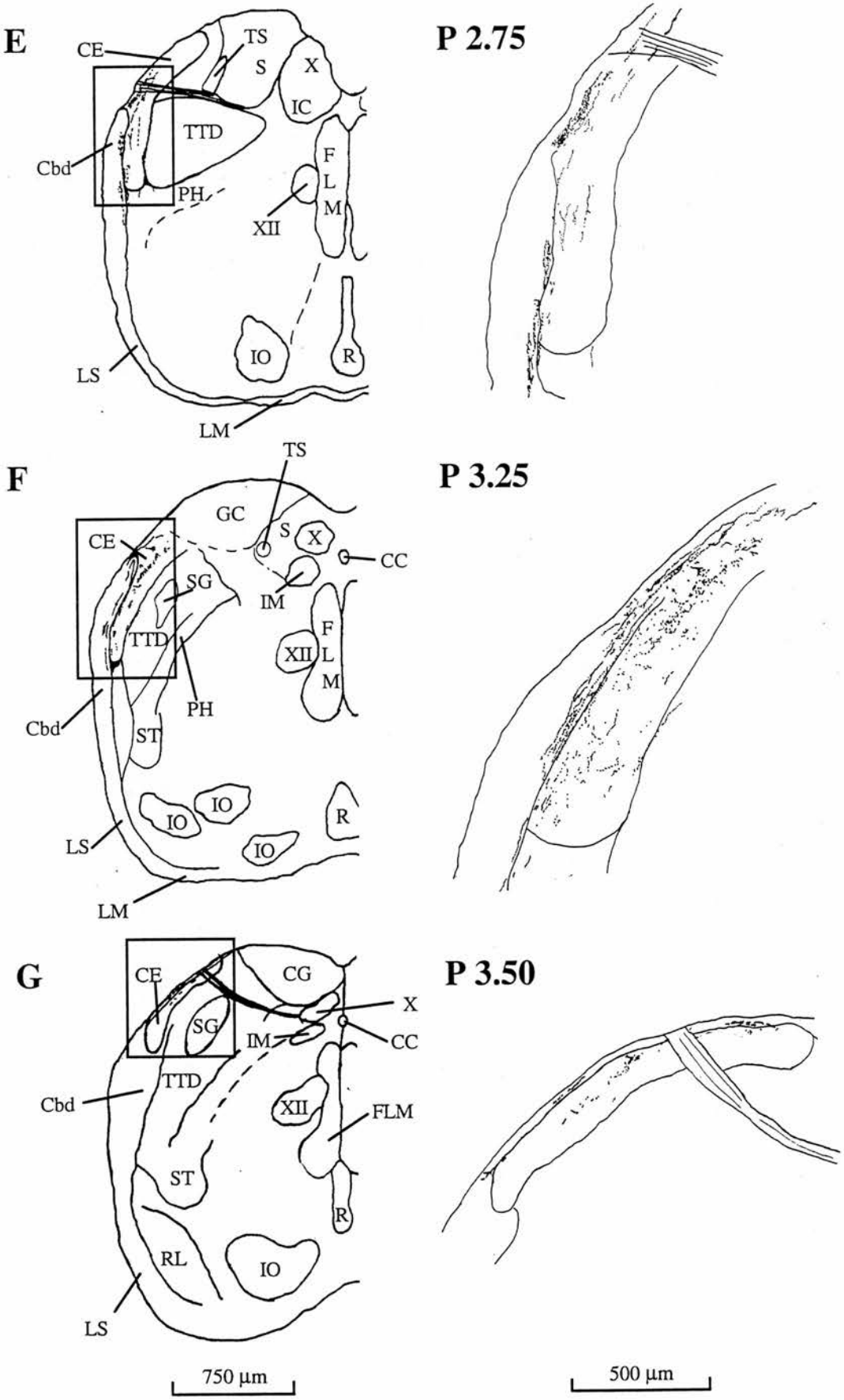
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4.4 DISCUSSION

The results of the present study are in agreement with the vast majority of anatomical studies using horseradish peroxidase (HRP) as a retrograde tracer and wheatgerm agglutinin conjugated to horseradish peroxidase (WGA/HRP) as an anterograde tracer on the anatomical substrate of extraocular muscle (EOM) proprioception. The results also confirm the presence of strongly labelled "medullary neurones" following the injection of HRP and WGA/HRP into selected EOM as described by Eden et al (1982), but show that this labelling is due to spread to the underlying quadratus and pyramidalis muscles innervated by the accessory abducens nucleus. This may also explain the authors' surprising control result where "one EOM was exposed but not injected. Surrounding orbital tissues were flooded with HRP. No intramedullary labeled (sic) neurones were noted." No information is given as to which EOM was exposed or whether labelled oculomotor neurones were found, but it is possible that spread to the underlying quadratus and pyramidalis muscles would not have been seen without injection into an EOM.

Primary afferent cell localization

These data demonstrate that the somata of the EOM afferents are confined to a restricted portion of the trigeminal ganglion in the pigeon, which would thus conform to the accepted pattern for the various mammalian species studied (Bortolami et al, 1987; Daunicht et al, 1985; Manni et al, 1976; Porter and Donaldson, 1991; Porter and Spencer, 1982) (Figure 4.2). While it was impossible to section the trigeminal ganglion in the same plane in each experiment, Figure 4.2 shows labelled axons in the ophthalmic nerve entering the trigeminal ganglion and labelled somata in a restricted region of the ganglion, suggesting that the proprioceptive neurones are localized in the ophthalmic subdivision of the trigeminal ganglion.

Projections to the external cuneate nucleus

The EOM afferent terminal projection to a discrete region of the external cuneate nucleus is a reproducible finding (Figure 4.4). Analysis of the location of the labelled terminals revealed specificity for the ventrolateral region of the nucleus. Fibres of passage were present in the incoming fibres of the trigeminal nerve and

within the rostrocaudal extent of the lateral spinal trigeminal nucleus (lTTD). Unlike the study of Buisseret-Delmas and Buisseret (1990) in the cat, no terminal label was found in the pars caudalis of the spinal trigeminal nucleus. This may reflect the relatively smaller amount of spread from WGA/HRP injections into EOM in the enclosed pigeon orbit compared to such injections into the EOM of the more open cat orbit. Similarly no terminal labelling was found in the caudal pars interpolaris of the spinal trigeminal nucleus, as has been found in the primate (Porter, 1986) and cat (Porter and Donaldson, 1991, Buisseret-Delmas and Buisseret, 1990). The lTTD contains trigeminal afferent fibres that project upon a ventral region of the external cuneate nucleus (Dubbeldam and Karten, 1978; Arends and Zeigler, 1989). It has not been described in mammals and is not found in other birds (e.g. the mallard, Arends and Dubbeldam, 1984), although it has been described in snakes (Molenaar, 1974 and Schroeder and Loop, 1976). It is interesting that the lTTD is not found in all snakes, but only in snakes possessing infrared sensitivity, particularly snakes such as pythons and rattlesnakes that possess facial and labial 'pits' which contain an infra-red sensitive surface comprised of densely packed non-overlapping trigeminal nerve endings. These 'warm' receptors enable the snake to locate and capture their homeothermic prey by detecting the prey's body heat. The morphology and bilateral position of these pits gives the opportunity for the extraction of spatial features from the afferent signals produced by these receptors. Evoked potentials from the pit receptors have been recorded in the optic tectum in close proximity to and in spatial register with visual units, further suggesting the spatial quality of the information produced by the infra-red receptors. Schroeder and Loop (1976) showed, using cobalt iontophoresis in the rattlesnake, that the afferent fibres from the maxillary branch of the trigeminal nerve which innervate the infra-red pit receptors travel within the lTTD and terminate solely in the nucleus of the lateral descending trigeminal tract (external cuneate nucleus). It is striking that trigeminal afferent fibres encoding spatial information in the snake are located in exactly the same position as the labelled terminals from presumptive proprioceptive neurones in the extraocular muscles of the pigeon. It is not difficult to imagine that spatial information gained from one sensory modality in the nearly blind snake is fulfilled by a different modality in the highly visual pigeon, and that the coincidence of the afferent distribution in the two animals is suggestive of a shared pathway for the processing of spatial information. The lack of a lateral spinal trigeminal nucleus in the cat or monkey suggests that afferent fibres from EOM proprioceptors have a different course in these animals and that the subsequent processing of the afferent signal may be somewhat different.

Section of the ophthalmic branch of the trigeminal nerve (Vo)

The absence of labelled neuronal cell somata following section of Vo, and the absence of any terminal label is strong evidence that the path of afferent fibres from sensory receptors within the EOM is through the oculomotor nerves proper within the orbit, but these fibres then cross to the ophthalmic nerve by means of anastomoses located in the vicinity of the cavernous sinus (Batini et al, 1975). These fibres are then presumed to enter the brainstem via the sensory root of the trigeminal nerve. The demonstration of responses to eye muscle stretch in the proximal part of the oculomotor nerve (Manni et al, 1984, 1989) has led to the suggestion that some afferent fibres, possibly originating from nociceptors, may re-cross to the oculomotor nerves prior to entering the brainstem. This suggestion cannot be confirmed by the present experiments, but the lack of any terminal label following Vo section prior to EOM injection with WGA/HRP suggests that the terminal label seen in the other experiments was due to afferent fibres that pass through the trigeminal ganglion.

The continuing presence of the medullary, multipolar neurones following Vo section provides further evidence that these cells are not first-order proprioceptive neurones as suggested by Eden et al (1982); rather they are likely to be motorneurones of the accessory abducens nucleus innervating the quadratus and pyramidalis muscles, a finding obviously confirmed by the injection of tracer into the quadratus muscle.

Functional considerations

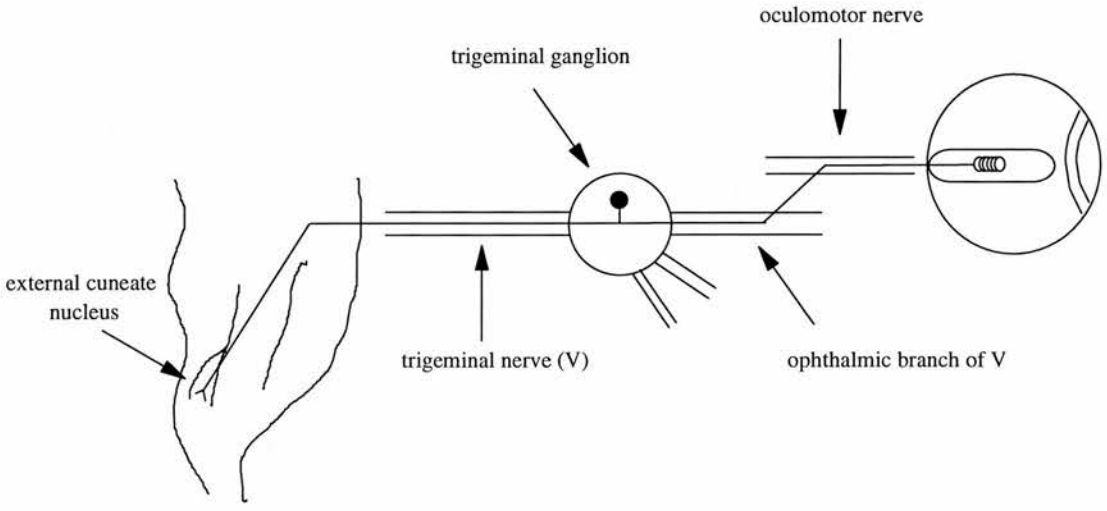
While an accurate account of the distribution of EOM proprioceptive information in the pigeon beyond the level of second-order afferent neurones in the external cuneate nucleus is not yet possible, EOM proprioceptive signals have been found in a number of visual and visuomotor regions (Donaldson and Knox, 1990a, Donaldson and Knox, 1991, Hayman et al, 1993a, b). Pathways to various elements of the vestibulo-oculomotor system may be via the cerebellum. The external cuneate nucleus projects to lateral parts of the ventral lamella of the inferior olive, which in turn supplies a portion of the anterior lobe of the cerebellar cortex (Arends and Zeigler, 1989).

Of relevance to the present findings is the fact that in mammals most skeletal muscles of the neck and upper extremity exhibit dual afferent representation involving the cuneate and lateral cuneate nuclei (Bakker et al, 1985; Edney and

Porter, 1986). Whereas Porter (1986) found labelled terminals solely in the pars triangularis of the cuneate nuclei and not in the external (lateral) cuneate nucleus of the monkey, pigeon EOM afferents terminate solely in the external cuneate nucleus.

The localization of EOM proprioceptive afferent terminals in a brainstem nucleus known to receive considerable inputs from neck muscle proprioceptors suggests that both signals may well be utilised in the control and stabilization of gaze. This is entirely consistent with the coordinated nature of gaze shifts, with both head and eyes being used to achieve a change in gaze, and the cooperative nature of the vestibular stabilization reflexes, the vestibuloocular and the vestibulocollic reflexes. An accurate representation of the direction of gaze (eye-in-space) requires signals proportional to the position of the eye in the head and of the head in space. Whilst the latter signal has long been thought to originate from neck muscle proprioceptors, the former (eye-in-head position) has generally been ascribed to internal copies of motor commands (corollary discharge). The present results suggest that EOM proprioceptive signals are also available to perform this task.

The pathway of EOM proprioception in the pigeon



CHAPTER 5. CONCLUSION

The preceding three chapters present complementary anatomical and electrophysiological results that clearly demonstrate the importance of afferent signals from the extraocular muscles in the control of gaze. Chapter two dealt with the use of EOM afferent signals in the control of head stabilization (the vestibulocollic reflex); Chapter three with the role afferent signals play in the control of eye movements and Chapter four the anatomical pathway of EOM proprioception. While the discussions within the individual chapters dealt with the points raised from each study, a number of more general points arise from all three studies and will be discussed hereafter. Furthermore, the review of literature on the study of extraocular muscle proprioception in Chapter one, suggests that many parallels can be made between the present results in the pigeon and many of the studies made in other animals, including Man.

Gaze control in the pigeon

The pigeon is not limited by a heavy head and seven cervical vertebrae as mammals are. This is reflected in the much greater use of its light, flexible head in visual and vestibular stabilization, particularly notable in the characteristic 'head bobbing' walk of the pigeon. The frequency response of the neck muscles reflects this greater head stabilization, with the phase of the response close to that seen in the extraocular muscles. This contrasts strongly with the situation in mammals, as described in Chapter two. The large and dramatic effects of imposed eye movement on the dorsal neck muscles of the pigeon may not be as easily compared to other animals as the results on the oculomotor system. This is simply because mammals use their eyes to stabilize gaze to a far greater extent than their heads. In Man, eliciting a vestibulocollic reflex is very difficult, although a strong vestibuloocular reflex is present. This is not to suggest that the results from the pigeon are not relevant to other species, just that in 'heavy-headed' animals the majority of gaze control is performed by the eyes and so the largest effects of extraocular muscle proprioception would be expected to be seen in the oculomotor system of mammals.

Notwithstanding this, the results found in pigeon neck muscles have their similarities to those found in other animals. The studies of Berthoz and co-workers on cats, monkeys and Man showed phasic and tonic modulations of neck muscle activity which were correlated to horizontal eye movements (see Chapter two).

While one hesitates to draw too many parallels between results gained in alert mammalian preparations and the decerebrate pigeon, the possibility that proprioceptive signals from the extraocular muscles are exerting some of the observed effects on neck muscles is surely worth investigating further, possibly by repeating the alert mammalian studies following section of the ophthalmic branch of the trigeminal nerve in these animals.

The role of the cerebellum in the proprioceptive control of eye movements remains unclear. The finding of Kimura and co-workers (1991) in the rabbit that lesions of the flocculus produced very similar effects on the VOR gain to those seen following section of the ophthalmic branch of the trigeminal nerve suggests that EOM afferent signals, which affect the VOR, pass through the flocculus. The presence of first-order EOM afferent terminals in the external cuneate nucleus of the pigeon, which is known to project to the cerebellum, suggests that the pathway of EOM proprioception in the pigeon beyond the first synapse may also pass through the cerebellum. The reduction in the gain of the VOR of the ipsilateral eye following section of the ophthalmic branch of the trigeminal nerve in the pigeon is very similar to the effect seen in the study of Kimura et al following a similar section, which again suggests that the findings of that study may be relevant to the pathway of EOM proprioception in the pigeon.

An important conclusion of Chapter two is that dorsal neck muscles respond to EOM afferent signals induced by IEM in specific ways which are dependent both on the particular neck muscle and the parameters of the imposed eye movement. Pairs of neck muscles (e.g. right and left *complexus* muscles) showed different, but complementary, responses to IEM which demonstrates the consistency and accuracy of the afferent signal. The quite different effects of IEM on a particular muscle in the three planes of vestibular stimulation and on different neck muscles during vestibular stimulation in a single plane indicate that the responses to IEM are related to a muscle's action. The fact that different muscles were affected quite differently by IEM shows that the EOM afferent signal produces muscle-specific effects rather than non-specific responses. This strongly suggests that the EOM afferent signal produced by IEM is a functionally significant input to the gaze control system.

The source of the afferent signal

The reasons for believing that imposed eye movement stimulates extraocular proprioceptors have already been discussed in Chapter two and will not be repeated here. However, it cannot be stated with complete certainty that imposed eye

movement does indeed produce afferent signals from the extraocular muscles. There are a number of, as yet, 'unknowns' that make this statement a strong assertion rather than fact. A major uncertainty is the lack of an identified sensory receptor in pigeon extraocular muscles to fulfil the proprioceptive role the present results suggest. It is known that avian extraocular muscles do not contain muscles spindles, the classical proprioceptor of most skeletal muscles (Maier et al, 1974). A likely candidate as proprioceptive end-organ in mammalian extraocular muscles is the musculotendinous cylinder or palisade ending (Alvarado-Mallart and Pinçon-Raymond, 1979; Ruskell, 1978) as described in Chapter one. This receptor has not yet been found in pigeon extraocular muscles. The fact that no-one has yet looked for the musculotendinous cylinder in pigeon extraocular muscles, should not, however, be taken as evidence against the presence of such a receptor. Indeed, the results of Chapters two and three on the effects of imposed eye movement on the maintenance of gaze in the pigeon provide circumstantial evidence which suggest the presence of precisely such a receptor.

One of the more striking results found with imposed eye movements on the vestibular reflexes in neck and extraocular muscles was that the largest effect on the electromyographic activity of the muscles (whether inhibitory or excitatory) was ALWAYS seen when an eye movement was imposed in one direction while the eye was attempting to move in the opposite direction, thereby stretching a contracting extraocular muscle. Ruskell (1978) suggested that because of its peculiar morphology, the musculotendinous cylinder would be preferentially stimulated by combined contraction of the small number of muscle fibres intimately associated with an individual receptor and stretch of the muscle. This is exactly the stimulus that produced the largest effect on neck or extraocular muscles. Lewis and Zee (1993) in their study on visual localization in a patient with trigeminal-oculomotor synkinesis (see Chapter 1), suggested that musculotendinous cylinders in human EOM,

"are more robustly activated with active muscle contraction than with passive muscle manipulation".

The similarity between the most effective stimulus in Man and pigeon is quite striking and provides further evidence that the afferent signal produced by imposed movement of the pigeon eye is similar to extraocular muscle proprioceptive signals seen in mammals and may well be produced by activation of musculotendinous cylinders in the extraocular muscles.

The possibility that the effects are purely due to these conditions (stretch + contraction) producing the largest noci- or mechanoreceptive stimulus from extra- and intra-orbital receptors is unlikely, as discussed in Chapter two. Furthermore, the

effect of imposed eye movement on the contralateral extraocular muscles during vestibular stimulation provides further evidence against this possibility. The largest effects of imposed eye movement in the neck musculature occurred soon after the start of imposed eye movement, but effects on the contralateral extraocular muscles were largest when eye movements were imposed half a second before the beginning of vestibularly-evoked activity in these muscles. It seems extremely unlikely that a non-proprioceptive afferent signal would produce effects on dorsal neck muscles with a latency of 50 milliseconds and yet not produce effects on the contralateral extraocular muscles for a further 450 milliseconds!

The differential effects produced by the latency of onset of imposed eye movement between neck and extra-ocular muscles is surprising. A possible explanation is that there are differences in the degree of control exerted by extraocular proprioception on neck muscles and contralateral extraocular muscles. The pigeon, unlike mammals, is able to make disconjugate eye movements. It is possible that the results reflect tight control of gaze with respect to the eye being moved, i.e. close correlation between ipsilateral eye and head movement, but looser coupling between the two eyes.

The effect of section of the ophthalmic branch of the Trigeminal nerve

Section of the ophthalmic branch of the Trigeminal nerve (Vo) produced quite striking effects on the movement of the ipsilateral eye and banished the effects of imposed movement of that eye. The removal of the effects of imposed eye movement following Vo section provide further confirmation that the effects of imposed eye movement are produced by stimulation of extraocular muscle proprioceptors. Section of Vo would also remove cutaneous, mechanoreceptive and nociceptive signals, since all these afferent fibres travel within Vo, but control experiments showed that these sensory modalities are not responsible for the effects of IEM (see Chapter two). The effect of Vo section on the ipsilateral eye certainly suggests that a vital signal has been removed. It seems incredible that the removal of relatively non-specific extraorbital afferent signals would disrupt the vestibuloocular reflex and the stability of the eye to the extent seen following Vo section.

Instability of the eye and disruption of the slow phase of the vestibuloocular reflex following Vo section have also been seen in the cat and rabbit; indeed, the effects of Vo section are almost identical in the three species. That three species with quite different repertoires of eye movements all show such similar effects following

section of a nerve believed by many neuroscientists to have no role in the control of eye movements strongly suggests that a fundamental signal has been removed, and that this signal is common to most, if not all, visual animals.

The absence of neuronal cell somata and brainstem terminals labelled with wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP) following Vo section strongly supports the conclusion that Vo section removes a functionally important afferent signal originating in the extraocular muscles. The injections of tracer into the extraocular muscles showed very little evidence of tracer spread outwith these muscles; corneal and nociceptive afferents are known to project to neurones interstitial to fibres of the spinal trigeminal tract in the cat (Chan-Palay, 1978; Marfurt, 1981 and Panneton and Burton, 1981) close to the ventrolateral border of the pars interpolaris of spinal trigeminal nucleus. No labelled terminals were noted in this region, nor within the pars caudalis of this nucleus which is also believed to receive nociceptive Trigeminal afferents (Porter, 1986). The presence of labelled terminals within the external cuneate nucleus, a brainstem nucleus which is known to receive neck muscle afferent fibres (Edney and Porter, 1986) provides an anatomical substrate for the observed interaction of extraocular muscle proprioceptive signals in the control of gaze.

It is interesting to note that Vo section produced very large disturbances in the slow phase of the VOR and the stability of the ipsilateral eye, but no discernible effect on the vestibular response of ipsilateral neck muscles, save the removal of any effect of imposed eye movement (Figure 2.42). The vestibulocollic reflex is known to be affected by proprioceptive signals from neck muscle spindles via the cervicocollic reflex (Dutia and Price, 1987) and to affect eye movements via the cervicoocular reflex. The effects of imposed eye movement on the vestibulocollic reflex suggest that a reflex analogous to the cervicoocular reflex exerts an influence on neck muscles, an oculocollic reflex perhaps.

Corollary discharge vs. EOM proprioception

The terms corollary discharge (Sperry, 1950) and efference copy (Von Holst and Mittelstaedt, 1950; Von Holst, 1954), while having quite distinct meanings, both arose from experiments involving surgical rotation of the eye (Sperry) or head (Von Holst and Mittelstaedt) which produced forced circling or 'a spontaneous optokinetic reaction'. Both authors rejected the possibility that their results were due to a reversal of the feedback signal in the optokinetic system, commenting that if this were so an animal would be the slave of its own optokinetic system and this was patently not

true, because the animal could move its eyes freely during normal orienting behaviour.

The rejection of a visual reflex as the source of the continuous circling following eye or head rotation appears very surprising in the light of current knowledge. It is now known that voluntary shifts in fixation can only be executed by the saccadic system, the velocity of these movements exceeding the operating range of the optokinetic system. Animals are unable to make slow, smooth eye movements against a stationary, featured background; in effect they are unable to make the voluntary shifts in gaze envisioned by Sperry and Von Holst and Mittelstaedt; hence it is questionable, even unlikely, that efference copy or corollary discharge signals produce, or are even available for the circling following eye or head rotation.

It appears that simple reversal of the feedback sign in the optokinetic system, producing positive feedback rather than a negative feedback stabilization system, with visual signals reinforcing rather than opposing eye movement, is sufficient to explain the circling that follows eye or head rotation.

Corollary discharge and efference copy have long been accepted as the most likely source of eye position information in oculomotor control and visual perception, as discussed in Chapter one. Yet little additional evidence beyond the original findings of Sperry and Von Holst and Mittelstaedt supporting their theories has been published. One of the few pieces of published work still supporting the stringent terms Von Holst and Mittelstaedt used to define efference copy is the study of Bell (1981) on the brain areas receiving afferent input from ampullary electroreceptors in a mormyrid fish.

There have been many demonstrations of central neural activity that is related to motor commands and is unlikely to have an afferent basis; however such activity has often erroneously been described as corollary discharge without evidence for its participation in perceptual processes, or as efference copy without evidence for the specific suppression of reafference, but not exafference.

Internal copies of motor commands within the visual system have been found by a number of researchers (Guthrie et al, 1983; Richmond & Wurtz, 1980; Robinson & Wurtz, 1976; Toyama et al, 1984). An interesting similarity between all these studies is that they were all studying saccadic eye movements. The results of the present study showed that EOM afferent signals produce striking effects on gaze stabilizing reflexes during vestibular stimulation.

It is possible that EOM afferent signals play a greater role in gaze stabilization than in gaze shifts. The presence of sensory receptors in the EOM of all

the species examined so far, species with a wide diversity of eye movements, supports the theory that EOM afferent signals play a fundamental role in eye movement control.

The lack of functional muscle spindles within the EOM of all species save the artiodactyl ungulates and the presence of the musculotendinous cylinder as the most probable proprioceptor suggests that the EOM contain a phylogenetically old form of proprioceptive sense organ, in parallel with the presence of multiply-innervated muscle fibres in the EOM, more commonly seen in reptiles and amphibia. This hypothesis would be consistent with the proprioceptive control of a system such as the VOR which, it appears, was one of the first types of eye movements to evolve (Carpenter, 1988) and is, therefore, seen in all the animals in which EOM sensory receptors have been found and in which responses to passive eye movement have been shown to affect the vestibular activity of single-units in brainstem areas involved in oculomotor control (Ashton et al, 1984b - toad; Ashton et al, 1989 - trout; Ashton et al, 1988 - cat; Donaldson and Knox, 1990a, 1993 - pigeon). Saccadic eye movements are found in animals with a high degree of ocular motility. The development of saccades in higher vertebrates may mean that different control systems, including the use of internal copies of motor commands, also developed.

Conclusions

The present results add further evidence to the growing literature on the effects of proprioception from the extraocular muscles on the control of gaze. The overwhelming finding of this literature is that afferent signals from the extraocular muscles play an important, if not fundamental, role in the moment-to-moment control of head and eye movements. It seems that it is time to reassess the role of extraocular muscle proprioception in gaze control in general. This could have considerable implications for our understanding of the various systems involved in visuomotor control and for the numerous models that have been constructed in an attempt to understand their operation. More importantly it may lead to a better understanding of human oculomotor control and pathology. Damage to the extraocular muscles, their afferent nerve supply and the trigeminal system could even be involved in visuomotor disorders such as strabismus.

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Effects of extraocular muscle afferent signals on the electromyogram of pigeon neck muscles during the vestibulo-collic reflex

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Afferent signals from extraocular muscle (EOM) afferents reach the vestibular nuclei, reticular formation and oculomotor nuclei of the pigeon (Donaldson & Knox, 1990, 1991) where they interact with vestibular signals and modify the electromyogram of the EOM during the vestibulo-ocular reflex (VOR) (Knox & Donaldson, 1991). We now show that EOM afferent signals also influence vestibulo-collic reflexes (VCR) in neck muscles involved in head stabilization (Dutia, 1991).

The electromyogram (EMG) of the left or right *complexus* or *rectus capitis*, recorded from pigeons decerebrated under ether anaesthesia, was rectified and averaged (Dutia & Hunter, 1985) during horizontal sinusoidal oscillation (frequencies 0.1 to 1.0 Hz) to induce the VCR. In interleaved trials during oscillation of the bird, movements were imposed on the left eye using a suction contact lens (Donaldson & Knox, 1990; Knox & Donaldson, 1991).

In some experiments neck muscle EMG was modulated sinusoidally during horizontal sinusoidal oscillation. More commonly, the EMG showed one to three bursts of increased activity as the head moved contralaterally. Imposed trapezoidal eye movements (5–20 deg at 115 deg s^{-1}) caused reduction of the vestibularly evoked EMG activity. The magnitude of this reduction was closely related to the amplitude of imposed eye movement decreasing by as much as 70 % for the largest eye movements used. Imposed sinusoidal eye movements that mimicked the slow phase of the VOR, and were thus compensatory in relation to the vestibular stimulus, reduced the EMG modulation during the VCR below that seen without eye movement. Imposed eye velocities greater than that required for compensation caused a proportional reduction in the vestibularly evoked EMG activity.

These results suggest that afferent signals from the EOM modulate not only the VOR but also the VCR and may thus have an important role in gaze stabilization and eye–head co-ordination.

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36.4

Extraocular muscle afferent signals affect the activity of neck muscles in the pigeon

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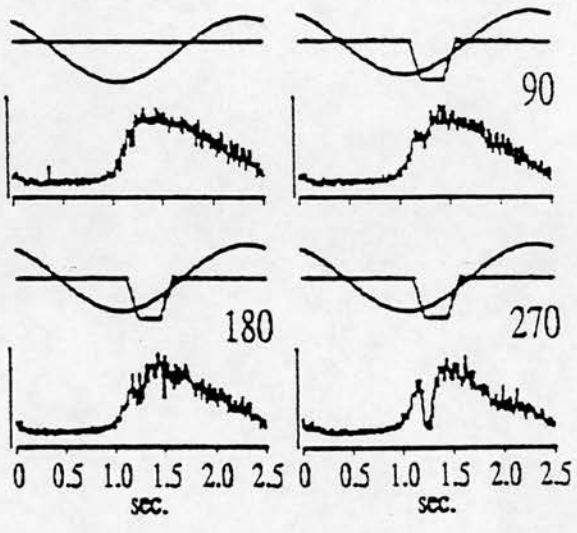
Movements of the head and eye which together result in changes in the direction of gaze are linked in a number of species, including man, with horizontal eye position being closely correlated to activity in dorsal neck muscles. The source of the eye position signal has generally been thought to be an internal motor copy of eye position (an efference copy).

Having recently shown that afferent signals from the extraocular muscles (EOM) modify the vestibuloocular reflex (VOR) of the pigeon we have now studied interactions between head and eye movements by recording the electromyographic activity of several neck muscles in the decerebrate pigeon during sinusoidal, horizontal, vestibular stimulation; such a stimulus evokes a vestibulocollic reflex (VCR) in neck muscles. Imposed movements of one eye (IEM) activated EOM stretch receptors and produced considerable modifications in the VCR response of a number of neck muscles. The magnitude of these effects was dependent on the parameters of the IEM such as the amplitude, velocity or direction of movement. The figure shows the effect of IEM towards the tail (90 deg.), vertically downwards (180 deg.) and towards the beak (270 deg.) on the VCR response of the ipsilateral *splenius capitis* muscle. Slow, sinusoidal, imposed eye movements that mimicked the slow phase of the VOR produced modifications in the VCR response which appeared to correct for errors in the imposed eye velocity and thus to maintain the direction of gaze.



The results show that EOM afferent signals have striking effects on the electromyographic activity of neck muscles during the VCR and strongly suggest that they are involved in head-eye coordination.

M.R.H. holds an SERC studentship. We are grateful to the Wellcome Trust and the W.H. Ross Foundation (Scotland) for support.

PROPRIOCEPTION AND EYE MOVEMENTS

◆ **Extraocular muscle afferent signals modify the activity of neck muscles during the vestibulo-collic reflex**

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Afferent signals from stretch receptors in neck muscles have long been known to affect head and eye movements. It is generally thought that stretch receptors in the extraocular muscles (EOM) do not serve a similar purpose, although neck-muscle activity shows a close correlation with eye position in a number of species. Having recently shown that EOM afferent signals do affect eye movements during the vestibulo-ocular reflex (VOR) we have now studied the effects of such signals on dorsal neck muscles during the vestibulo-collic reflex (VCR).

The electromyogram (EMG) of the left or right complexus or splenius capitis, recorded from adult pigeons decerebrated under ether anaesthesia, was rectified and averaged during horizontal sinusoidal oscillation to induce the VCR. In interleaved trials during oscillation of the bird, movements were imposed on the left eye by means of a suction contact lens. Imposed eye movements at saccadic velocities produced considerable modifications in the VCR response of both neck muscles. Movement of the eye in the opposite direction to that produced by the VOR produced large inhibitions in the VCR response, whereas movements in the same direction as the VOR produced only modest inhibitions in the VCR response of the muscles tested. This effect may be due to a combination of active contraction of EOM and imposed stretch producing the largest afferent signal from EOM stretch receptors. Slow sinusoidal imposed eye movements that mimicked the slow phase of the vestibulo-ocular reflex produced modifications of the VCR response which appear to correct for errors in the imposed eye velocity and thus to maintain the direction of gaze. The results show that changes in eye position have striking effects on the EMG activity of neck muscles during the VCR and strongly suggest that EOM afferent signals are involved in head–eye coordination and gaze stabilisation.

Afferent signals from pigeon extraocular muscles modify the activity of neck muscles during the vestibulocollic reflex

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SUMMARY

Movements of the head and eye which, together, result in changes in the direction of gaze are linked in a number of species, including man, and eye position is known to affect the activity of neck muscles. This head–eye linkage has generally been ascribed to modification of neck muscle activity by internal estimates of eye position derived from motor commands. We have recently shown that afferent signals from stretch receptors in the extraocular muscles are involved in the moment-to-moment control of eye movements during the vestibuloocular reflex (VOR). We have now studied the interactions between head and eye movements by recording the electromyographic activity of several neck muscles during horizontal (yaw) or frontal (roll tilt) vestibular stimulation. Such a stimulus evokes a VOR in the eyes and a vestibulocollic reflex (VCR) in neck muscles. Imposing movements on one eye at saccadic velocities produced considerable inhibition of the VCR response of a number of neck muscles. The magnitude of these effects was dependent on the parameters of the imposed eye movement. Thus systematic changes were seen when the amplitude, velocity or direction of eye movement was varied. Movement of the eye in the opposite direction to that produced by a normal VOR produced a large inhibition of the VCR response, whereas movements in the same direction as the VOR produced only modest inhibition of the VCR response of the neck muscles tested. Slow, sinusoidal, imposed eye movements that mimicked the slow phase of the VOR produced changes in the gain of the VCR response which appear to correct for errors in the imposed eye velocity and thus tend to maintain the direction of gaze. The results show that changes in eye position have striking effects on the electromyographic activity of neck muscles during the VCR, and strongly suggest that extraocular muscle afferent signals are involved in head–eye coordination.

1. INTRODUCTION

Coordinated control of the head and eye is required for stabilizing and shifting the direction of gaze. Head movement stimulates the vestibular system producing stabilizing reflexes in the head and eyes. The vestibuloocular reflex (VOR) produces compensatory eye movements to stabilize the eyes in space, and the vestibulocollic reflex (VCR) resists and counteracts head movement, stabilizing the head in space.

During intentional head movements (gaze shifts), the VCR and VOR are modulated or suppressed when they antagonize the orienting behaviour (Bizzi 1981; Guitton 1988). Vidal *et al.* (1982) showed modulation of the VCR in alert cats that was closely correlated with horizontal eye position. The VCR was completely suppressed or facilitated depending upon whether the eye was directed to the contralateral or to the ipsilateral side of the orbit.

Although neck muscle proprioceptive signals are known to affect the gain of both the VCR (Dutia & Hunter 1985) and the VOR (Barmack *et al.* 1989), afferent signals from extraocular muscle (EOM) stretch receptors have generally not been considered to be involved in the reflex control of eye or head movements (Carpenter 1988). The reasons for this have been discussed in detail previously (Donaldson & Knox 1990).

However, evidence has recently accumulated that proprioceptive feedback from the EOM does affect eye movements. Chronic deafferentation of the EOM produces disruption of the slow phase of the VOR in rabbits (Kashii *et al.* 1989) and instability of cat eye movements in the dark (Fiorentini & Maffei 1977). We have shown that afferent signals from EOM in the decerebrate pigeon reach the vestibular and oculomotor nuclei and the reticular formation (Donaldson & Knox 1990; Donaldson & Knox 1991) and, recently (Knox & Donaldson 1993), that imposed movements of one eye considerably modify movements of the contralateral eye during the VOR, as measured by the electro-oculogram (EOG). These results strongly suggest that afferent signals from stretch receptors in the EOM affect oculomotor control. The latter experiments also suggest that this control may be exercised from moment to moment.

Internal estimates of eye position derived from motor commands have generally been believed to explain head–eye coupling during gaze shifts (Grantyn *et al.* 1987; Vidal *et al.* 1982), although André-Deshays *et al.* (1988) suggested that afferent signals from the extraocular muscles (EOM) might play a partial role in

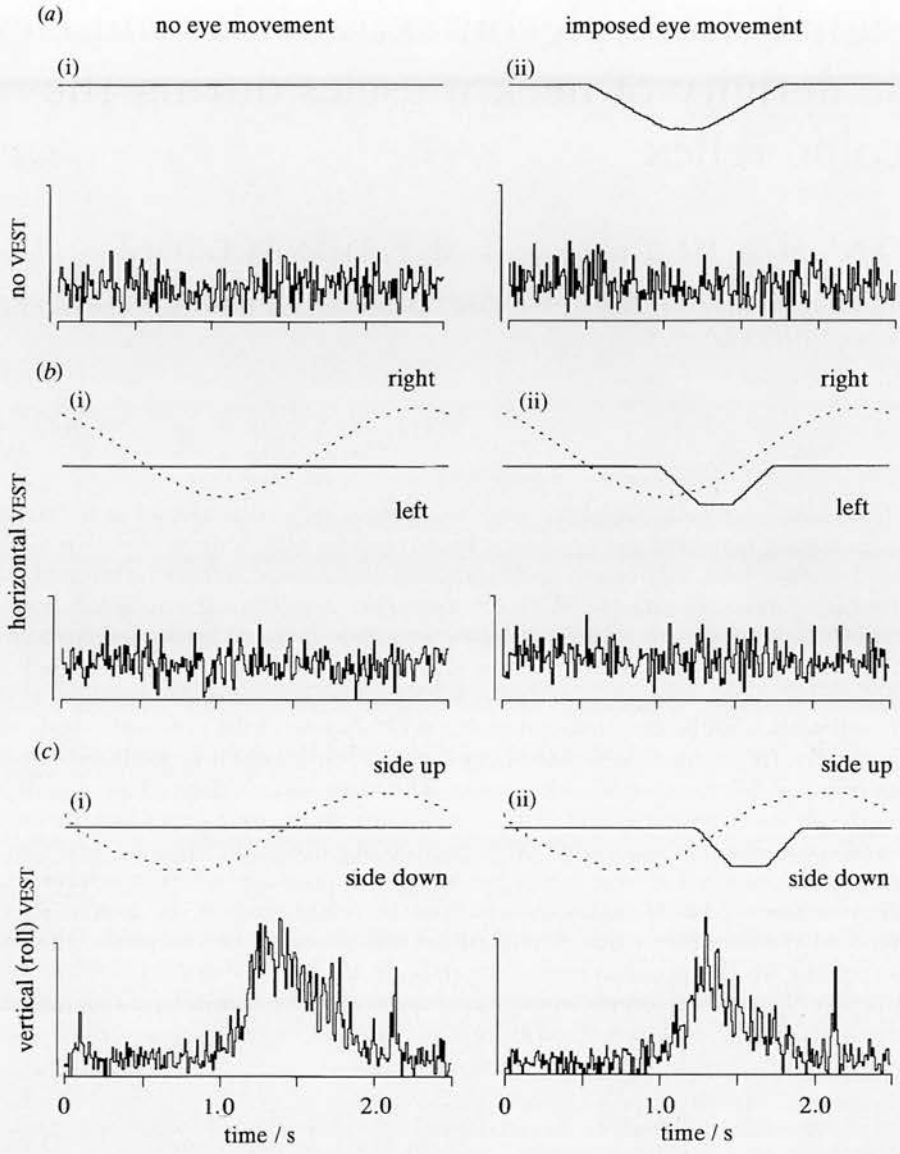

Figure 1. Effect of vestibular stimulation (VEST) (no eye movement) (i) and imposed eye movement (IEM) (ii) on the electromyogram (EMG) of the ipsilateral biventer cervicis muscle. Six cycle histograms (CHSTS) showing rectified EMG data averaged over 24 sweeps. (*a*) Spontaneous activity unaffected by IEM. (*b*) VEST in the horizontal plane produces no effect on the spontaneous activity of the left biventer cervicis muscle with or without IEM of the left eye. (*c*) VEST in the frontal plane (roll tilt) produces a vestibulocollic reflex in the right biventer cervicis muscle which is inhibited by IEM of the right eye. Head position shown by broken sinusoid ($\pm 8°$), eye position shown by solid trapezoid. Scale bars, 6.4 μV.

this coupling. The aim of the experiments described here was to investigate whether EOM afferent signals produced by imposed eye movements affect the activity of neck muscles during vestibular stimulation. A preliminary report of these results has appeared (Hayman *et al.* 1993).

2. METHODS

All the results described were obtained in decerebrate pigeons. The preparation and techniques of recording were identical to those described previously (Knox & Donaldson 1991). Natural vestibular stimulation (VEST) in the horizontal plane (yaw) was produced as described previously (Donaldson & Knox 1990). For VEST in the frontal plane (roll tilt), the bird was placed on a platform attached to a servo-controlled direct current printed-circuit motor (G16M4 Printed Motors Ltd, Bordon, Hants) with the headholder positioned so that the axis of rotation passed through the centre of the atlanto-occipital joint.

During sinusoidal oscillation (usually $\pm 8°$ at 0.4 Hz), one eye (the left eye for horizontal VEST and the right for frontal VEST) was moved by an electromagnetic servo-controlled device that acted upon a stalk carried by an opaque contact lens held firmly to the cornea and sclera by suction (Ashton *et al.* 1984). The eye-mover device attached to the horizontal turntable could be rotated to cause movement in any desired orbital plane (e.g. horizontal, vertical, diagonal), whereas the device attached to the frontal turntable was limited to movements in the vertical plane. Local anaesthetic (lignocaine 1%) was routinely applied to the cornea before application of the lens and at regular intervals thereafter.

Two types of imposed eye movement (IEM) were used: (i) trapezoidal, pseudosaccadic (amplitude 15°, velocity $115° s^{-1}$), in which the movement started from the centre of

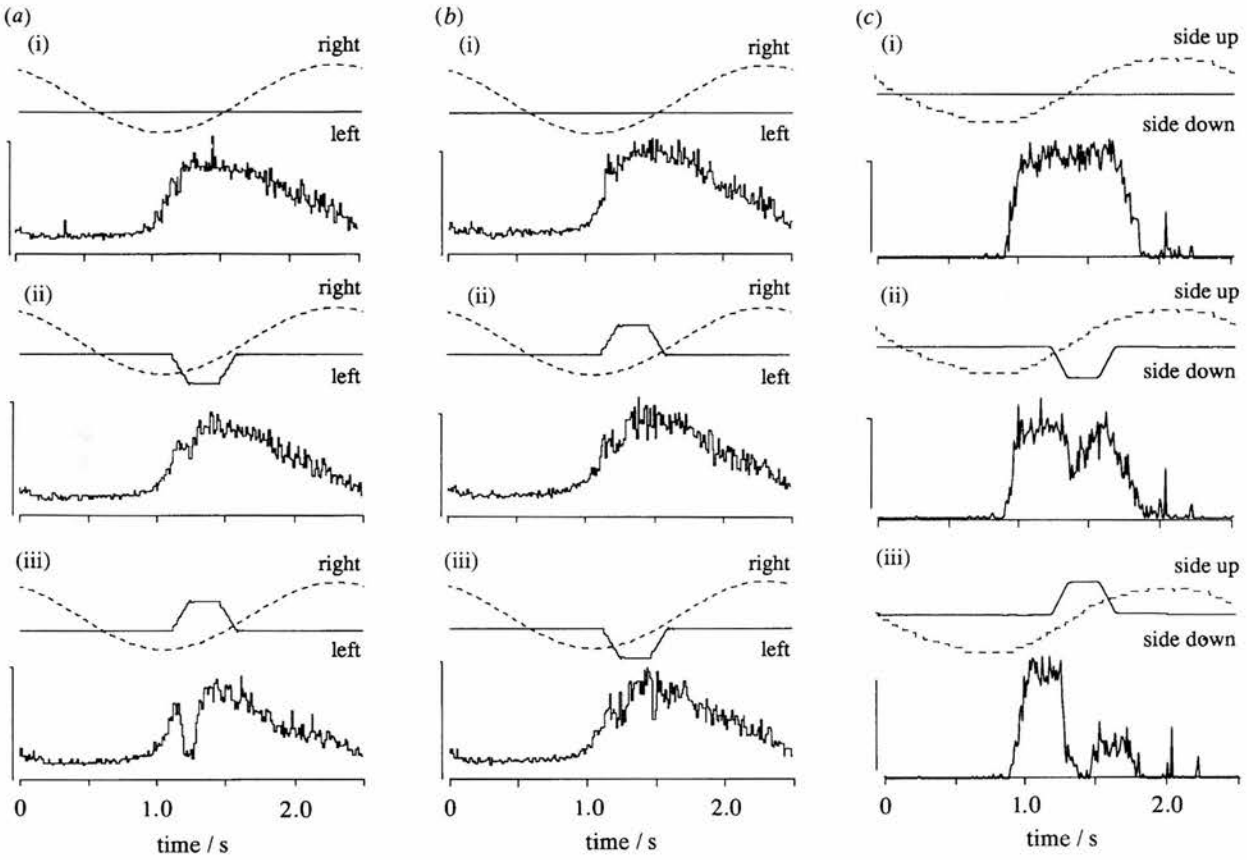

Figure 2. Effect of different directions of imposed eye movement (IEM) at saccadic velocities on the vestibulocollic reflex response of the left splenius capitis and right complexus muscles. (*a*) Cycle histograms (CHSTS) showing rectified EMG data from the left splenius capitis muscle averaged over 24 sweeps during horizontal vestibular stimulation (yaw). (i) Vestibulocollic reflex (VCR) response when there was no imposed movement of the left eye; (ii) effect of IEM (15° at 115° s^{-1}) directed towards the left (tail); (iii) effect of IEM towards the right (beak). (*b*) CHSTS of the VCR response of the left splenius capitis muscle, again during horizontal vestibular stimulation (yaw). (i) Control CHST; (ii) effect of imposed movement of the left eye vertically upwards; (iii) effect of IEM vertically downwards. (*c*) (i) VCR response of the right complexus muscle during vestibular stimulation in the frontal plane (roll tilt) when there was no imposed movement of the right eye; (ii) effect of IEM vertically downwards (amplitude 15°, velocity 115° s^{-1}) on the VCR response; (iii) effect of IEM vertically upwards on the VCR response. Head position is shown by broken sinusoid (±8°), eye position shown by solid trapezoid. Scale bars, 6.4 μV (yaw), 15.6 μV (roll).

the orbit then moved out along a particular radius at constant velocity to a new position, at which it was held for 200 ms, before retracing its path to the centre; and (ii) sinusoidal in timecourse, thus mimicking the slow phase of the VOR, always at the same frequency as the vestibular turntable but with varying phases or velocities to produce variants of an 'artificial VOR' (AVOR); when the eye was moved passively at the same speed as the table but in the opposite direction (180° out of phase), this stimulus was called the compensatory AVOR.

The four neck muscles tested were identified by dissection and with reference to an atlas of avian anatomy (Chamberlain 1943). Complexus is the most superficial dorsal neck muscle in the pigeon; it has a large insertion onto the occipital crest, and its action is to extend the neck dorsally and laterally. Rectus capitis dorsalis major lies deep to complexus and biventer cervicis. It is similar to splenius capitis in most mammals (George & Berger 1966), and will henceforth be described as splenius capitis. Its action is to extend the head on the neck. Biventer cervicis lies bilaterally in the midline deep to complexus, and its action is to flex the head dorsally. Rectus capitis ventralis lateralis lies lateral to complexus, inserting on the nuchal surface of the occipital bone, its action being ventral and lateral flexion of the head on the neck.

Bipolar silver wire electrodes were placed on one or two of the right or left complexus, splenius capitis, biventer cervicis or rectus capitis ventralis lateralis muscles. Multi-unit electromyograms (EMG) were recorded, differentially amplified (amplification 10000–50000), filtered (pass-band 30 Hz to 10 kHz) and fed to the analogue-to-digital converter of the computer used to control the experiment and collect the data, a CED 1401 Programmable Interface (CED, Cambridge, England), controlled from an IBM-AT compatible microcomputer.

The collection and analysis of EMG data was identical to that described by Knox & Donaldson (1991). Briefly, four or eight cycle histograms (CHST), interleaved in time, of rectified and averaged EMG activity, were collected over several (usually 24) complete cycles of vestibular stimulation (Donaldson & Knox 1991). Sinusoids of the frequency used for vestibular stimulation were fitted to the records of table position and to the CHSTS of the vestibularly driven EMG by using a modification of the method of Arzi & Magnin (1989). In some experiments the modulation of the EMG signal by the stimulus was calculated by subtracting from each bin of the record the average background voltage during the part of the cycle when the muscle was not active. Where the phase of the 'artificial VOR' was varied (see figure 5), a phase of zero was taken to be the phase that produces the compensatory AVOR (i.e. 180° out of phase with the table).

3. RESULTS

The effect of horizontal and frontal (roll) vestibular stimulation on the dorsal neck muscles of 40 pigeons was studied. Three of the four muscles studied, complexus, splenius capitis and rectus capitis ventralis lateralis, responded sometimes during only a portion of the vestibular cycle. In other preparations, muscles displayed resting EMG activity which was modulated sinusoidally throughout the cycle in response to vestibular stimulation. Both types of reflex response were found in all muscles tested. The biventer cervicis muscle did not exhibit reflex activation in response to vestibular stimulation in the horizontal plane, but vestibular stimulation in the frontal plane did produce a strong VCR response (figure 1*b*, *c*).

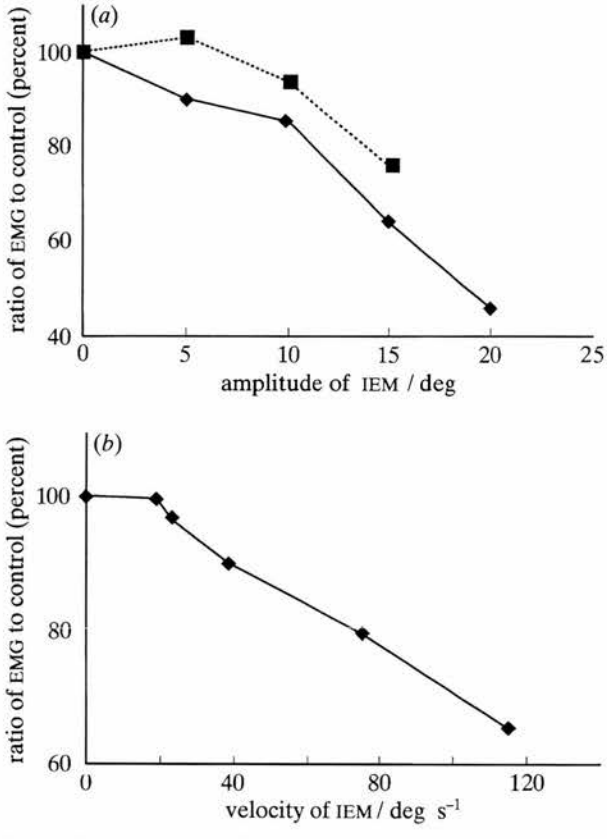

Figure 3. (*a*) Effect of change of amplitude (5°–20°) of horizontal imposed movement of the left eye towards the left (tail) (squares) or right (beak) (diamonds), velocity held constant at 115° s^{-1}, on the left splenius muscle. The graph shows the ratio of the modulation of the averaged EMG during combined horizontal, sinusoidal, vestibular stimulation and IEM, to the modulation of the EMG during vestibular stimulation alone (control), plotted against the amplitude of the IEM (see text for further details). The magnitude of the reduction in EMG modulation is dependent on the direction of the eye movement, as well as on its amplitude with movements towards the right (beak) having the largest effect. (*b*) Effect of change of velocity of horizontal IEM towards the beak, amplitude held constant at 15°, on the horizontal VCR of the left splenius muscle. The graph shows the ratio of the modulation of the averaged EMG during combined horizontal, sinusoidal vestibular stimulation and the initial segment of the eye movement, to the modulation of the EMG during vestibular stimulation alone (control), plotted against the velocity of the IEM (see Methods for further details).

(*a*) *Effect of imposed eye movement* (IEM) *alone*

Without vestibular stimulation there was no effect of IEM on any of the muscles tested, whether spontaneous activity was present or not (figure 1*a*).

(*b*) *Effect of imposed eye movement* (IEM) *and vestibular stimulation*

The effect of IEM on the horizontal and frontal VCR was investigated by using trapezoidal eye movements (amplitude 15° at 115° s^{-1}) along a number of orbital radii. The biventer cervicis muscle was unaffected by IEM during vestibular stimulation in the horizontal plane, although IEM during frontal vestibular stimulation did produce inhibition of the VCR response (figure 1*c*). The horizontal and frontal VCR responses of splenius capitis, rectus capitis ventralis lateralis and complexus were consistently inhibited by IEM, and the magnitude of the effect depended upon the direction of the initial eye movement. When the initial eye movement was in the opposite direction to table movement there was little inhibition of the VCR response. Thus movement of the eye to the left as the table moved to the right (figure 2*a*) produced a small inhibition of the horizontal VCR response. Similarly, movement of the eye vertically downwards as the table moved vertically upwards produced a small inhibition of the frontal VCR response (figure 2*c*). Conversely, when the IEM was in the same direction as table movement, the inhibition of the VCR response was large (figure 2*a*, *c*). Imposed eye movements in the vertical plane during horizontal vestibular stimulation produced little or no effect on the VCR response (figures 2*b* and 4).

Varying the amplitude of the trapezoidal IEM from 5° to 20° produced systematic effects on the modulation of the VCR responses; Figure 3*a* shows that there were reductions of up to 50 % of control values (using no IEM as the control) for the largest amplitudes used. A similar, systematic reduction was found when the amplitude of IEM was held constant at 15° and the velocity was altered from 19° to 115° s^{-1} (figure 3*b*). The effects of varying the amplitude or velocity of the IEM were also dependent on the direction of the eye movement. The magnitude of the inhibition was greatest when the IEM was directed in the same direction as the table movement.

Figure 4 shows the effect of different directions of trapezoidal IEM on the ipsilateral and contralateral complexus muscles during vestibular stimulation in the horizontal plane. The effect of IEM is 'directionally tuned'. The VCR response of the left complexus (ipsilateral) is inhibited to the greatest extent by IEM towards the beak while the table moves to the right. The right complexus (contralateral) is inhibited by IEM towards the tail while the table moves to the left. Thus IEM produces the greatest inhibition of the VCR when eye and head move in the same direction. The effect of IEM on the contralateral muscle was consistently much weaker than on the ipsilateral muscle. This was true whether the left or right eye was moved.

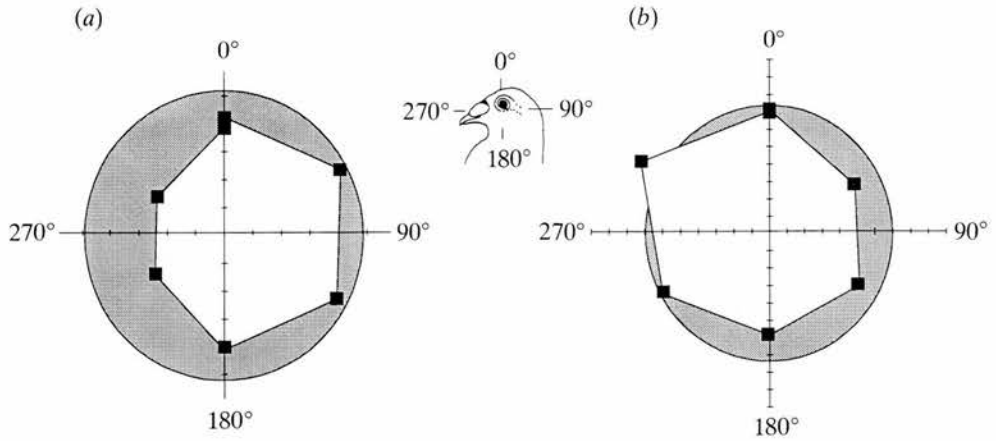

Figure 4. Plots of 'directional-tuning' of the effects of imposed movements of the left eye on the VCR response of (*a*) the left complexus (ipsilateral) and (*b*) the right complexus (contralateral). The plots are derived from sets of eight interleaved EMG recordings during horizontal vestibular stimulation alone (control) and with added imposed eye movement in various directions: 0° vertical plane, upwards; 90° horizontal plane, towards tail; 180° vertical plane, downwards; and 270° horizontal plane towards beak. The plots were constructed from the modulation of the rectified EMG integrated over a time window in each record. The responses are plotted as vectors in which the distance of a point from the centre of the plot represents the magnitude of the response, and the angle of the vector shows the direction of imposed eye movement (IEM). The shaded area shows the inhibition produced by IEM relative to the control (no eye movement) VCR response. (*a*) Circle, no eye movement; scale divisions, 0.5 μV; response window bars 147–226 (800 ms). (*b*) Circle, no eye movement; scale divisions, 1.0 μV; response window bars 131–175 (450 ms).

(*c*) *Effect of artificial VOR*

By using slow sinusoidal IEM, the 'artificial VOR', the effect of perturbations of eye movement over the whole vestibular stimulus cycle could be investigated. Altering the phase of the imposed sinusoid produced a position error. With increasing phase lag there was a systematic reduction in the gain of the VCR response of all the muscles, compared with that at the compensatory AVOR; decreases of up to 40% were observed (figure 5). A small increase in the VCR gain was found with increasing phase lead. The results obtained were very similar in both horizontal and frontal planes.

Figure 6 shows the effect of velocity errors produced by altering the velocity of the imposed sinusoid while maintaining the same frequency as that of the table. When the imposed peak eye velocity was below that of the compensatory AVOR, peak velocity (usually 22° s^{-1}), the gain of the vestibular response was increased. In some experiments increases of up to 40% of the VCR response at the compensatory VOR were observed. When the eye velocity exceeded that needed for compensation the gain was decreased; decreases of up to 50% of the VCR response at the compensatory AVOR were observed.

4. DISCUSSION

The results of these experiments clearly show that EOM afferent signals produced by imposed eye movements of one eye do affect the VCR responses of several dorsal neck muscles. This extends our previous results, which have investigated the effects of EOM afferent signals on the oculomotor system, to a different, although closely linked, motor system, that of the head and neck.

The evidence that the effective signal during imposed movement of the eye arises from the extraocular muscle proprioceptors has been presented and discussed in several of our previous reports (see Donaldson & Knox 1990), and so will not be repeated here.

(*a*) *Nature of interactions during pseudosaccadic IEM*

Whereas splenius capitis, complexus and rectus capitis ventralis lateralis all showed a VCR in the horizontal and frontal planes which was inhibited by IEM, the biventer cervicis muscle was only affected by vestibular stimulation and IEM in the frontal plane (figure 1*c*). This suggests that the action of IEM may be specific to the plane of action of the particular neck muscle. It agrees with the observation in the cat that eye position signals reflected in neck muscle activity with the head fixed correlate with the preferred plane of neck muscle activity with the head free (Roucoux *et al.* 1989). Our finding that, during vestibular stimulation in the horizontal plane, vertical IEM has very little effect on the VCR response (figure 2*b*) argues that IEM predominantly affects neck muscles when the eye movement is in the same plane as that of the vestibular stimulation.

A notable feature of the response to IEM was that no effect was seen on the resting EMG activity in any of the muscles tested. It was not until the bird was being oscillated that IEM produced modulations in those muscles with a VCR response (figure 1). Possibly it is only the vestibular drive to neck muscles with which the EOM afferent signal interacts. The effect of pseudosaccadic eye movement on the VCR response showed 'directional tuning'. Table movement in one direction (for example, to the right) evokes a compensatory VOR activating EOM to move the eye in the opposite direction (to the left). The IEMs that produced the greatest inhibition in the VCR response of the neck

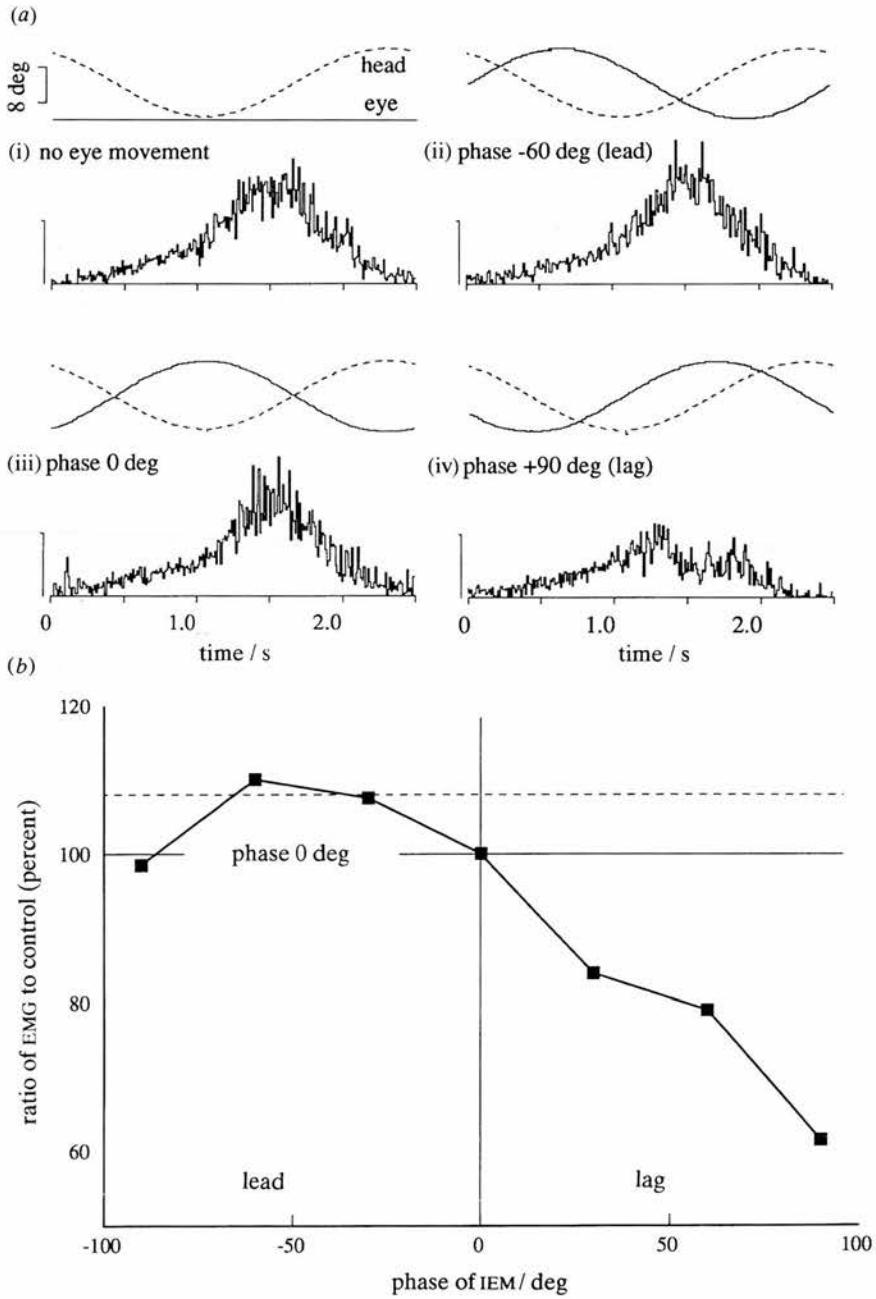

Figure 5. Effect of varying the phase of 'artificial VOR' on VCR activity of the left splenius muscle. (*a*) Four CHSTs from an interleaved set of eight, showing the averaged EMG during the VCR response of the left splenius muscle; (i) no IEM; (ii) IEM at compensatory velocity for vestibular stimulus, but with a phase lead of 60°; (iii) at compensatory VOR; (iv) at compensatory velocity, but with a phase lag of 90°. Broken line shows head position, solid line shows eye position. Scale bars, 2.56 μV. (*b*) Results from same experiment as (*a*). Plot of the ratio of the VCR response of the left splenius muscle during imposed movements of the left eye with various phases (±90°), to its VCR response at the compensatory VOR (phase 0°). The VCR response is decreased with increasing phase lag, and increased with increasing phase lead. The broken line is the VCR response when there was no eye movement (see text for further details).

muscles were directed in the same direction as table movement, and therefore in the opposite direction to the compensatory eye movement which the VOR produces, i.e. the IEM stretched the EOM in one direction while they were contracting to move the eye in the opposite direction. Thus, during horizontal oscillation, IEMs towards the beak when the EOM were contracting in an attempt to move the eye towards the tail produced larger inhibitions than IEM towards the beak (figures 2*a* and 4). Corresponding effects were found in the vertical plane (figure 2*c*).

Phasic inhibitions during horizontal saccades towards the contralateral side are seen in ipsilateral dorsal neck muscles in man (André-Deshays *et al.* 1991) and cat (Vidal *et al.* 1982). Thus the directional effects of IEMs at saccadic velocities on dorsal neck muscles during the VCR appear qualitatively similar to those seen during spontaneous saccades.

Of course, we do not know whether the directional effects are due to this peripheral interaction of stretch and muscle contraction or to central interactions (or both). Previous studies have shown that passive eye

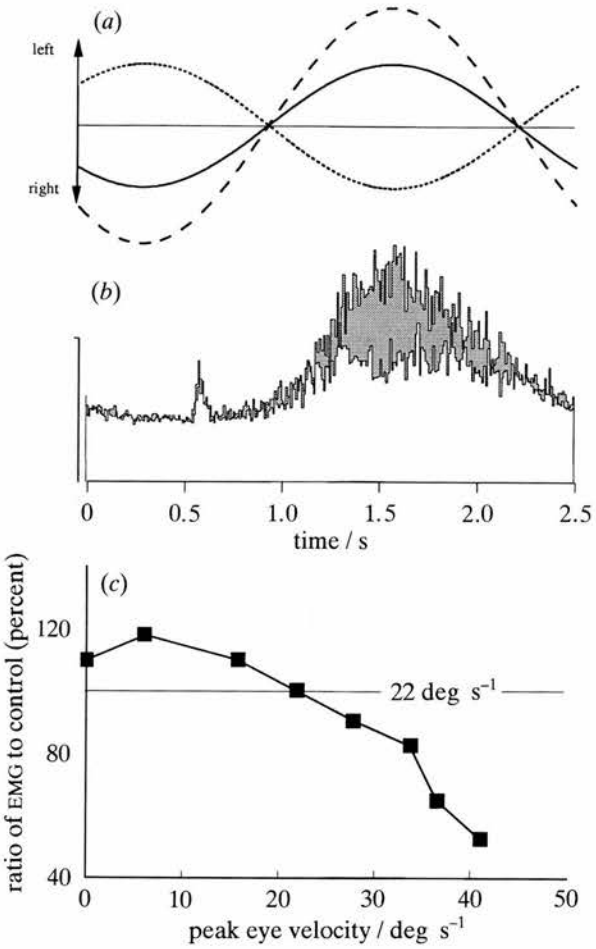

Figure 6. Experiment using 'artificial VOR' with velocity errors as explained in text. (*a*) Head velocity during a vestibular stimulus cycle at 0.4 Hz, peak velocity, 22° s^{-1} (dotted line); eye velocity during compensatory aVOR (solid line); and eye velocity at 41° s^{-1} (dashed line). (*b*) Superposed records of EMG modulation of the left splenius muscle during the 'artificial VOR' from a set of interleaved histograms averaged over 24 repetitions. The shaded area represents the reduction in the VCR response in splenius when the imposed eye velocity is almost twice that required to produce a compensatory VOR. Scale bar, 6.4 μV. (*c*) Results from the same experiment as (*b*). Plot of the ratio of the VCR response of the left splenius muscle during imposed movements of the left eye at various velocities, to its VCR response during the compensatory VOR (eye velocity, 22° s^{-1}). The VCR response is reduced as eye velocity increases above the 22° s^{-1} required for compensation of the vestibular stimulus, and increases as eye velocity decreases below that required for compensation (see text for further details).

movement affects the vestibular modulation of single units in directionally specific ways in the brain stem of paralysed pigeons (Donaldson & Knox 1990).

The effects of IEM on neck muscles on the side opposite (contralateral) to that of the eye moved by the suction contact lens were weak (figure 4). Studies of head–eye co-ordination have emphasized the ipsilateral nature of the eye–head linkage observed (André-Deshays *et al.* 1991). This is a further example of the similarity between the results of studies of eye–head linkage in intact preparations and the effects of EOM afferent signals during the VCR of the decerebrate pigeon.

(*b*) *Interactions during the artificial VOR*

The artificial VOR, in mimicking the slow phase of the VOR, is presumably producing EOM afferent signals that more closely represent the natural situation than do the signals produced by pseudosaccadic IEM. The effects of the artificial VOR complement those seen using pseudosaccadic eye movements; errors of velocity or direction (phase) produce effects similar to those seen with pseudosaccadic eye movements. However, unlike the phasic modifications produced by pseudosaccadic eye movements, eye movement errors produced by the artificial VOR affect the whole of the VCR response. Longer-lasting (tonic) effects of eye movement on neck muscle activity have also been seen during natural eye movements (André-Deshays *et al.* 1988).

The artificial VOR with phase errors provides a further example of the effect that different directions of IEM have on the VCR response of neck muscles. The VOR is stimulated by table rotation; as the table begins to rotate, EOM motorneurons discharge to move the eyes in the opposite direction. An aVOR with a phase lead moves the eye ahead of the motorneuron drive, 'unloading' the muscles that are contracting. An aVOR with a phase lag moves the eye against the motorneuron drive, stretching the contracting muscles. As was seen with IEM at pseudosaccadic velocities, the combination of EOM contraction and stretch produced the greatest inhibition of the VCR response (figure 5, phase lag). 'Unloading' the EOM (figure 5, phase lead) produced a slight increase in the VCR response of the neck muscles studied relative to the VCR response at the compensatory aVOR. The VCR response without eye movement was always greater than the response at the compensatory aVOR.

The results obtained by using the artificial VOR with velocity errors suggest that the EOM afferent signal has a corrective effect on the VCR response (figure 6). Movements of the eye at velocities below the compensatory VOR increased the gain of the VCR. If the head were free to move, an increased VCR gain would limit head movement to correspond to the reduced eye movement, and this would tend to produce a stable gaze direction. Conversely, when the eye velocity exceeded that needed for compensation, the gain of the VCR response was reduced. This, again, would tend to stabilize the direction of gaze if the head were free to move. However, this corrective hypothesis cannot easily be extended to cover the results obtained by using fast (pseudosaccadic) eye movements. See Donaldson & Knox (1993) for discussion of a 'difference-in-principle' between pseudosaccadic and slow sinusoidal IEMs.

An important result of the present study is that the phase of the VCR response is unaffected by the artificial VOR with velocity or phase errors, whereas the gain of the reflex is considerably modulated in both cases. This is in agreement with results from single unit recordings in areas of the pigeon brainstem involved in the control of gaze (Donaldson & Knox 1993).

(c) *Head–eye linkage*

Gioanni (1988) studied the VOR (head fixed) and closed-loop VCR (head free) in the alert pigeon and concluded that 'oculo-collic coupling is absent or very weak in the pigeon'. This is not consistent with the present results. However, the eye–head (oculo-collic) coupling described in alert cats, rabbits, monkeys and man (André-Deshays *et al.* 1988; Fuller 1980; Lestienne *et al.* 1984; Vidal *et al.* 1982) was observed in the EMG of dorsal neck muscles with the head fixed. Gioanni's (1988) study of the pigeon's VCR was made with the head free, and the methods used were unlikely to reveal eye–head coupling as only gross head movement was studied, not individual muscle activity.

It is clear from the present results that imposed movements of one eye have striking effects upon the VCR response of dorsal neck muscles in the pigeon, and that these results show remarkable similarities to the effects of natural eye movements on the VCR response of dorsal neck muscles in several species. The interaction between the parameters of the IEM in producing various degrees of inhibition of the VCR response in neck muscles shows that EOM afferent signals convey specific information to neck muscles about eye movement. This strongly suggests that EOM afferent signals are involved in eye–head coordination and thus may play an important role in the control of gaze.

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